MIXTURES OF GROWTH HORMONE DRUG-OLIGOMER CONJUGATES COMPRISING POLYALKYLENE GLYCOL, USES THEREOF, AND METHODS OF MAKING SAME

Field Of The Invention

The present invention relates to drug-oligomer conjugates, and, more particularly, to growth hormone drug-oligomer conjugates.

Background Of The Invention

Growth hormones have been used for replacement therapy in growth hormone-deficient children. Growth hormone is also under investigation as an adjunct in the treatment of several catabolic conditions such as burn injuries, surgery and malabsorption. The positive effects of growth hormone on calcium ion retention and on osteogenesis also may be of use in the treatment of osteoporosis and non-healing fractures.

Growth hormone may be administered by intramuscular or subcutaneous injection. Of these methods, subcutaneous injection may be preferred because it facilitates self-administration. However, both of these methods may be less than optimal because of the psychological and/or physical trauma that may be associated with administration by injection, especially in children.

Pharmaceutically active molecules such as proteins and polypeptides have been conjugated with polydispersed mixtures of polyethylene glycol or polydispersed mixtures of polyethylene glycol containing polymers to provide polydispersed mixtures of drug-oligomer

conjugates. For example, U.S. Patent No. 5,359,030 to Ekwuribe proposes conjugating polypeptides such as somatostatin, somatotropin and/or somatomedin with polydispersed polyethylene glycol modified glycolipid polymers and polydispersed polyethylene glycol modified fatty acid polymers. The number average molecular weight of polydispersed polymer resulting from each combination is preferred to be in the range of from about 500 to about 10,000 Daltons.

Polyethylene glycol (PEG) is typically produced by base-catalyzed ring-opening polymerization of ethylene oxide. The reaction is initiated by adding ethylene oxide to ethylene glycol, with potassium hydroxide as a catalyst. This process results in a polydispersed mixture of polyethylene glycol polymers having a number average molecular weight within a given range of molecular weights. For example, PEG products offered by Sigma-Aldrich of Milwaukee, Wisconsin are provided in polydispersed mixtures such as PEG 400 (M_n 380-420); PEG 1,000 (M_n 950-1,050); PEG 1,500 (M_n 1,400-1,600); and PEG 2,000 (M_n 1,900-2,200).

It is desirable to provide non-polydispersed mixtures of growth hormone-oligomer conjugates where the oligomer comprises polyalkylene glycol.

Summary Of The Invention

A mixture of growth hormone-oligomer conjugates comprising polyalkylene glycol according to embodiments of the present invention may exhibit higher *in vivo* activity than a polydispersed mixture of similar conjugates, where the polydispersed mixture has the same number average molecular weight as the mixture according to the present invention. This heightened activity may result in lower dosage requirements. Moreover, a mixture of growth hormone-oligomer conjugates comprising polyalkylene glycol according to embodiments of the present invention may be more effective at surviving an *in vitro* model of intestinal digestion than polydispersed mixtures of similar conjugates. Furthermore, mixtures of growth hormone-oligomer conjugates comprising polyalkylene glycol according to embodiments of the present invention may also result in less inter-subject variability than polydispersed mixtures of similar conjugates.

According to embodiments of the present invention, a substantially monodispersed mixture of conjugates where each conjugate includes a growth hormone drug coupled to an oligomer that comprises a polyalkylene glycol moiety is provided. The polyalkylene glycol

moiety preferably has at least 2, 3, or 4 polyalkylene glycol subunits and, most preferably, has at least 7 polyalkylene glycol subunits. The polyalkylene glycol moiety is preferably polypropylene glycol. The oligomer preferably further comprises a lipophilic moiety. The growth hormone drug is preferably human growth hormone. The oligomer is preferably covalently coupled to an amino function of the human growth hormone. The conjugate is preferably amphiphilically balanced such that the conjugate is aqueously soluble and able to penetrate biological membranes. The oligomer may comprise a first polyalkylene glycol moiety covalently coupled to the drug by a non-hydrolyzable bond and a second polyalkylene glycol moiety covalently coupled to the first polyalkylene glycol moiety by a hydrolyzable bond. The mixture is preferably a monodispersed mixture and is most preferably a purely monodispersed mixture.

According to other embodiments of the present invention, a substantially monodispersed mixture of conjugates is provided where each conjugate comprises a growth hormone drug coupled to an oligomer including a polyalkylene glycol moiety, and the mixture has an *in vivo* activity that is greater than the *in vivo* activity of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture.

According to still other embodiments of the present invention, a substantially monodispersed mixture of conjugates is provided where each conjugate comprises a growth hormone drug coupled to an oligomer including a polyalkylene glycol moiety, and the mixture has an *in vitro* activity that is greater than the *in vitro* activity of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture.

According to other embodiments of the present invention, a substantially monodispersed mixture of conjugates is provided where each conjugate comprises a growth hormone drug coupled to an oligomer including a polyalkylene glycol moiety, and the mixture has an increased resistance to degradation by chymotrypsin when compared to the resistance to degradation by chymotrypsin of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture.

According to yet other embodiments of the present invention, a substantially monodispersed mixture of conjugates is provided where each conjugate comprises a growth

hormone drug coupled to an oligomer including a polyalkylene glycol moiety, and the mixture has an inter-subject variability that is less than the inter-subject variability of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture.

According to other embodiments of the present invention, a mixture of conjugates is provided where each conjugate includes a growth hormone drug coupled to an oligomer that comprises a polyalkylene glycol moiety, and the mixture has a molecular weight distribution with a standard deviation of less than about 22 Daltons.

According to still other embodiments of the present invention, a mixture of conjugates is provided where each conjugate includes a growth hormone drug coupled to an oligomer that comprises a polyalkylene glycol moiety, and the mixture has a dispersity coefficient (DC) greater than 10,000 where

$$DC = \frac{\left(\sum_{i=1}^{n} N_i M_i\right)^2}{\sum_{i=1}^{n} N_i M_i^2 \sum_{i=1}^{n} N_i - \left(\sum_{i=1}^{n} N_i M_i\right)^2}$$

wherein:

n is the number of different molecules in the sample;

N_i is the number of $i^{\underline{th}}$ molecules in the sample; and

M_i is the mass of the $i^{\underline{th}}$ molecule.

According to other embodiments of the present invention, a mixture of conjugates is provided in which each conjugate includes a growth hormone drug coupled to an oligomer and has the same number of polyalkylene glycol subunits.

According to still other embodiments of the present invention, a mixture of conjugates is provided in which each conjugate has the same molecular weight and has the formula:

$$\text{Growth Hormone Drug}\left[B - L_h - G_i - R_m - G'_j - R'_n - G''_k - T\right]_p \qquad (A)$$

wherein:

B is a bonding moiety;

L is a linker moiety;

G, G' and G" are individually selected spacer moieties;

R is a lipophilic moiety and R' is a polyalkylene glycol moiety, or R' is the lipophilic moiety and R is the polyalkylene glycol moiety;

T is a terminating moiety;

h, i, j, k, m and n are individually 0 or 1, with the proviso that when R is the polyalkylene glycol moiety; m is 1, and when R' is the polyalkylene glycol moiety, n is 1; and

p is an integer from 1 to the number of nucleophilic residues on the growth hormone drug.

Methods of synthesizing mixtures of the various embodiments are also provided by the present invention.

Pharmaceutical compositions comprising conjugate mixtures of the present invention are also provided. Methods of treating a growth hormone deficiency in a subject in need of such treatment by administering an effective amount of such pharmaceutical compositions are also provided.

Methods of accelerating the growth rate of an animal by administering to the animal an effective amount of a mixture of conjugates according to the various embodiments described above are also provided.

Growth hormone drug-oligomer conjugate mixtures according to embodiments of the present invention may provide increased *in vivo* activity and/or lowered inter-subject variability and/or decreased degradation by chymotrypsin when compared to conventional polydispersed growth hormone drug-oligomer conjugate mixtures.

Brief Description of the Drawings

Figure 1 illustrates a generic scheme for synthesizing a mixture of activated polymers comprising a polyethylene glycol moiety and a fatty acid moiety according to embodiments of the present invention;

Figure 2 illustrates a scheme for synthesizing a mixture of mPEG according to embodiments of the present invention;

Figure 3 illustrates a scheme for synthesizing a mixture of activated mPEG7-hexyl oligomers according to embodiments of the present invention;

Figure 4 illustrates a scheme for synthesizing a mixture of activated mPEG7-octyl oligomers according to embodiments of the present invention;

Figure 5 illustrates a scheme for synthesizing a mixture of activated mPEG-decyl oligomers according to embodiments of the present invention;

Figure 6 illustrates a scheme for synthesizing a mixture of activated stearate-PEG6 oligomers according to embodiments of the present invention;

Figure 7 illustrates a scheme for synthesizing a mixture of activated stearate-PEG8 oligomers according to embodiments of the present invention;

Figure 8 illustrates a scheme for synthesizing a mixture of activated PEG3 oligomers according to embodiments of the present invention;

Figure 9 illustrates a scheme for synthesizing a mixture of activated palmitate-PEG3 oligomers according to embodiments of the present invention;

Figure 10 illustrates a scheme for synthesizing a mixture of activated PEG6 oligomers and conjugating human growth hormone with the activated PEG6 oligomers according to embodiments of the present invention;

Figure 11 illustrates a scheme for synthesizing various propylene glycol monomers according to embodiments of the present invention;

Figure 12 illustrates a scheme for synthesizing various propylene glycol polymers according to embodiments of the present invention;

Figure 13 illustrates a scheme for synthesizing various propylene glycol polymers according to embodiments of the present invention;

Figure 14 is an HPLC trace (HPLC gradient: 50% to 90% acetonitrile in 30 minutes) of the conjugation reaction illustrated in **Figure 10** using two equivalents of activated MPEG6 oligomers and five equivalents of activated MPEG6 oligomers;

Figure 15 is an HPLC trace (HPLC gradient: 0% to 95% acetonitrile in 20 minutes) of the conjugation reaction illustrated in **Figure 10** using 30 equivalents of activated MPEG6 oligomers;

Figure 16 is a MALDI spectra of the conjugation reaction illustrated in **Figure 10** using two equivalents of activated MPEG6 oligomers;

Figure 17 is an HPLC trace (HPLC gradient: 50% to 70% acetonitrile in 30 minutes) illustrating a partial purification of the product of the conjugation reaction of **Figure 10** using five equivalents of activated MPEG6 oligomers;

Figure 18 is a MALDI spectra of fraction B from the partial purification illustrated in **Figure 17**;

Figure 19 is a MALDI spectra of fraction C from the partial purification illustrated in **Figure 17**;

Figure 20 is a MALDI spectra of fractions D and E from the partial purification illustrated in **Figure 17**;

Figure 21 is an electrospray spectra of fraction E from the partial purification illustrated in **Figure 17**;

Figure 22 is an electrospray spectra of the reaction mixture from the conjugation reaction illustrated in **Figure 10** using 30 equivalents of activated MPEG6 oligomers;

Figure 23 is an HPLC trace of a conjugation reaction of human growth hormone with the activated oligomer of **Figure 9**;

Figure 24 is an HPLC trace of a conjugation reaction using one equivalent of human growth hormone and two equivalents of the activated oligomer of **Figure 9**;

Figure 25 is an HPLC trace of a conjugation reaction using one equivalent of human growth hormone and five equivalents of the activated oligomer of **Figure 8**;

Figure 26 is a MALDI spectra of the fraction corresponding to the left half of the peak in the conjugation HPLC trace of **Figure 25**;

Figure 27 is a MALDI spectra of the fraction corresponding to the right half of the peak in the conjugation HPLC trace of **Figure 25**;

Figure 28 is an HPLC trace of a conjugation reaction using one equivalent of human growth hormone and nine equivalents of the activated oligomer of **Figure 8**;

Figure 29 illustrates a bar graph denoting the activity as determined by luciferase assay of mixtures of growth hormone conjugates according to embodiments of the present invention compared with the activity of human growth hormone standards, which are provided for comparison purposes only and do not form part of the present invention; and

Figure 30 illustrates a bar graph denoting the activity as determined by luciferase assay of mixtures of growth hormone conjugates according to embodiments of the present invention compared with the activity of human growth hormone standards, which are provided for comparison purposes only and do not form part of the present invention.

Detailed Description Of Preferred Embodiments

The invention will now be described with respect to preferred embodiments described herein. It should be appreciated however that these embodiments are for the purpose of

illustrating the invention, and are not to be construed as limiting the scope of the invention as defined by the claims.

As used herein, the term "non-polydispersed" is used to describe a mixture of compounds having a dispersity that is in contrast to the polydispersed mixtures described in U.S. Patent No. 5,359,030 to Ekwuribe.

As used herein, the term "substantially monodispersed" is used to describe a mixture of compounds wherein at least about 95 percent of the compounds in the mixture have the same molecular weight.

As used herein, the term "monodispersed" is used to describe a mixture of compounds wherein about 100 percent of the compounds in the mixture have the same molecular weight.

As used herein, the term "substantially purely monodispersed" is used to describe a mixture of compounds wherein at least about 95 percent of the compounds in the mixture have the same molecular weight and have the same molecular structure. Thus, a substantially purely monodispersed mixture is a substantially monodispersed mixture, but a substantially monodispersed mixture is not necessarily a substantially purely monodispersed mixture.

As used herein, the term "purely monodispersed" is used to describe a mixture of compounds wherein about 100 percent of the compounds in the mixture have the same molecular weight and have the same molecular structure. Thus, a purely monodispersed mixture is a monodispersed mixture, but a monodispersed mixture is not necessarily a purely monodispersed mixture.

As used herein, the term "weight average molecular weight" is defined as the sum of the products of the weight fraction for a given molecule in the mixture times the mass of the molecule for each molecule in the mixture. The "weight average molecular weight" is represented by the symbol M_w.

As used herein, the term "number average molecular weight" is defined as the total weight of a mixture divided by the number of molecules in the mixture and is represented by the symbol M_n.

As used herein, the term "dispersity coefficient" (DC) is defined by the formula:

$$DC = \frac{\left(\sum_{i=1}^{n} N_i M_i\right)^2}{\sum_{i=1}^{n} N_i M_i^2 \sum_{i=1}^{n} N_i - \left(\sum_{i=1}^{n} N_i M_i\right)^2}$$

wherein:

n is the number of different molecules in the sample;

N_i is the number of i^{th} molecules in the sample; and

M_i is the mass of the i^{th} molecule.

As used herein, the term "intra-subject variability" means the variability in activity occurring within the same subject when the subject is administered the same dose of a drug or pharmaceutical composition at different times.

As used herein, the term "inter-subject variability" means the variability in activity between two or more subjects when each subject is administered the same dose of a given drug or pharmaceutical formulation.

As used herein, the term "growth hormone drug" means a drug possessing all or some of the biological activity of growth hormone peptides.

As used herein, the term "growth hormone peptides" means human growth hormone, human growth hormone-releasing hormone, animal growth hormone or animal growth hormone-releasing hormone any of which may be provided by natural, synthetic, or genetically engineered sources.

As used herein, the term "growth hormone peptide analog" means growth hormone peptide wherein one or more of the amino acids have been replaced while retaining some or all of the activity of the growth hormone peptide. The analog is described by noting the replacement amino acids with the position of the replacement as a superscript followed by a description of the growth hormone. For example, "Pro^{41} growth hormone, human" means that the amino acid typically found at the 41 position of a human growth hormone molecule has been replaced with proline.

Growth hormone analogs may be obtained by various means, as will be understood by those skilled in the art. For example, certain amino acids may be substituted for other amino acids in the growth hormone structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. As the interactive capacity and nature of growth hormone defines its biological functional activity, certain amino acid sequence substitutions can be made in the amino acid sequence and nevertheless remain a polypeptide with like properties.

In making such substitutions, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive

biologic function on a polypeptide is generally understood in the art. It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant polypeptide, which in turn defines the interaction of the polypeptide with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics as follows: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5). As will be understood by those skilled in the art, certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a polypeptide with similar biological activity, *i.e.,* still obtain a biological functionally equivalent polypeptide. In making such changes, the substitution of amino acids whose hydropathic indices are within ±2 of each other is preferred, those which are within ±1 of each other are particularly preferred, and those within ±0.5 of each other are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent 4,554,101 provides that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (±3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); seine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). As is understood by those skilled in the art, an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent polypeptide. In such changes, the substitution of amino acids whose hydrophilicity values are within ±2 of each other is preferred, those which are within ±1 of each other are particularly preferred, and those within ±0.5 of each other are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the

relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions (i.e., amino acids that may be interchanged without significantly altering the biological activity of the polypeptide) that take various of the foregoing characteristics into consideration are well known to those of skill in the art and include, for example: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

As used herein, the term "growth hormone peptide fragment" means a segment of the amino acid sequence found in the growth hormone that retains some or all of the activity of the growth hormone polypeptide.

As used herein, the term "growth hormone peptide fragment analog" means a segment of the amino acid sequence found in the growth hormone peptide wherein one or more of the amino acids in the segment has been replaced while retaining some or all of the activity of the growth hormone polypeptide.

As used herein, the term "polyalkylene glycol" refers to straight or branched polyalkylene glycol polymers. The term “polyalkylene glycol subunit” refers to a single polyalkylene glycol unit. For example, a polyethylene glycol subunit is —(CH_2CH_2O)—.

As used herein, the term "lipophilic" means the ability to dissolve in lipids and/or the ability to penetrate, interact with and/or traverse biological membranes, and the term, "lipophilic moiety" or "lipophile" means a moiety which is lipophilic and/or which, when attached to another chemical entity, increases the lipophilicity of such chemical entity. Examples of lipophilic moieties include, but are not limited to, alkyls, fatty acids, esters of fatty acids, cholesteryl, adamantyl and the like.

As used herein, the term "lower alkyl" refers to substituted or unsubstituted alkyl moieties having from one to five carbon atoms.

As used herein, the term "higher alkyl" refers to substituted or unsubstituted alkyl moieties having six or more carbon atoms.

In embodiments of the present invention, a substantially monodispersed mixture of growth hormone drug-oligomer conjugates is provided. Each growth hormone drug-oligomer conjugate in the monodispersed mixture includes a growth hormone drug coupled to an oligomer that comprises a polyalkylene glycol moiety. Preferably, at least about 96, 97, 98 or 99 percent of the conjugates in the mixture have the same molecular weight. More preferably, the mixture is a monodispersed mixture. Even more preferably, the mixture is a

substantially purely monodispersed mixture. Still more preferably, at least about 96, 97, 98 or 99 percent of the conjugates in the mixture have the same molecular weight and have the same molecular structure. Most preferably, the mixture is a purely monodispersed mixture.

The growth hormone drug is preferably human growth hormone. However, it is to be understood that the growth hormone drug may be selected from various growth hormone drugs known to those skilled in the art including, for example, growth hormone peptides, growth hormone peptide analogues, growth hormone peptide fragments, and growth hormone peptide fragment analogues. Growth hormone peptides include, but are not limited to, growth hormone, human (hGH); growth hormone, porcine; growth hormone, bovine; growth hormone, chicken; growth hormone, rat; growth hormone, mouse; growth hormone, ovine; growth hormone releasing factor, human; growth hormone pro-releasing factor, human; growth hormone releasing factor, mouse; growth hormone releasing factor, ovine; growth hormone releasing factor, rat; growth hormone releasing factor, bovine; growth hormone releasing factor, porcine; and growth hormone releasing factor, chicken. Growth hormone peptide analogs may be provided as described above by substituting one or more amino acids in a growth hormone peptide. Growth hormone peptide fragments include, but are not limited to, growth hormone 1-43, human; growth hormone 6-13; growth hormone releasing factor 1-37, human; growth hormone releasing factor 1-40, human; growth hormone releasing factor 1-40, amide, human; growth hormone releasing factor 30-44, amide, human; growth hormone releasing factor 1-29, amide, rat; hexarelin (growth hormone releasing hexapeptide); and growth hormone releasing factor 1-29, amide, human. Growth hormone peptide fragment analogues include, but are not limited to, [D-Ala^{2}]-growth hormone releasing factor 1-29, amide, human; [N-Ac-Tyr^{1}, D-Arg^{2}]-growth hormone releasing factor 1-29, amide; [His^{1}, Nle^{27}]-growth hormone releasing factor 1-32, amide; growth hormone releasing peptide-6 ([His^{1}, Lys^{6}]-GHRP); and [D-Lys^{3}]-GHRP-6.

The oligomer may be various oligomers comprising a polyalkylene glycol moiety as will be understood by those skilled in the art. Preferably, the polyalkylene glycol moiety has at least 2, 3, or 4 polyalkylene glycol subunits. More preferably, the polyalkylene glycol moiety has at least 5 or 6 polyalkylene glycol subunits. Most preferably, the polyalkylene glycol moiety of the oligomer has at least 7 polyalkylene glycol subunits. The polyalkylene glycol moiety of the oligomer is preferably a lower alkyl polyalkylene glycol moiety such as a polyethylene glycol moiety, a polypropylene glycol moiety, or a polybutylene glycol

moiety. The polyalkylene glycol moiety is more preferably a polypropylene glycol moiety having a uniform structure. An exemplary polypropylene glycol moiety having a uniform structure is as follows:

$$-O-\underset{CH_3}{\underset{|}{CH}}-CH_2-O-\underset{CH_3}{\underset{|}{CH}}-CH_2-O-\underset{CH_3}{\underset{|}{CH}}-CH_2-O-\underset{CH_3}{\underset{|}{CH}}-CH_2-O-$$

This uniform polypropylene glycol structure may be described as having only one methyl substituted carbon atom adjacent each oxygen atom in the polypropylene glycol chain. Such uniform polypropylene glycol moieties may exhibit both lipophilic and hydrophilic characteristics and thus be useful in providing amphiphilic growth hormone drug-oligomer conjugates without the use of lipophilic polymer moieties. Furthermore, coupling the secondary alcohol moiety of the polypropylene glycol moiety with a growth hormone drug may provide the growth hormone drug (e.g., human growth hormone) with improved resistance to degradation caused by enzymes such as trypsin and chymotrypsin found, for example, in the gut.

Uniform polypropylene glycol according to embodiments of the present invention is preferably synthesized as illustrated in **Figures 11** through **13**, which will now be described. As illustrated in **Figure 11**, 1,2-propanediol **53** is reacted with a primary alcohol blocking reagent to provide a secondary alcohol extension monomer **54**. The primary alcohol blocking reagent may be various primary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, silylchloride compounds such as t-butyldiphenylsilylchloride and t-butyldimethylsilylchloride, and esterification reagents such as Ac_2O. Preferably, the primary alcohol blocking reagent is a primary alcohol blocking reagent that is substantially non-reactive with secondary alcohols, such as t-butyldiphenylsilylchloride or t-butyldimethylsilylchloride. The secondary alcohol extension monomer (**54**) may be reacted with methanesulfonyl chloride ($MeSO_2Cl$) to provide a primary extension alcohol monomer mesylate **55**.

Alternatively, the secondary alcohol extension monomer **54** may be reacted with a secondary alcohol blocking reagent to provide compound **56**. The secondary alcohol blocking reagent may be various secondary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, benzyl chloride. The compound **56** may be reacted with a B_1 de-blocking reagent to remove the blocking moiety B_1 and provide a primary alcohol extension monomer **57**. The B_1 de-blocking reagent may be selected from

various de-blocking reagents as will be understood by one skilled in the art. When the primary alcohol has been blocked by forming an ester, the B_1 de-blocking reagent is a de-esterification reagent, such as a base (e.g., potassium carbonate). When the primary alcohol has been blocked using a silylchloride, the B_1 de-blocking reagent is preferably tetrabutylammonium fluoride (TBAF). The primary alcohol extension monomer **57** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension monomer mesylate **58**.

The primary alcohol extension monomer **54** and the secondary alcohol extension monomer **57** may be capped as follows. The secondary alcohol extension monomer **54** may be reacted with a capping reagent to provide a compound **59**. The capping reagent may be various capping reagents as will be understood by those skilled in the art including, but not limited to, alkyl halides such as methyl chloride. The compound **59** may be reacted with a B_1 de-blocking agent as described above to provide a primary alcohol capping monomer **60**. The primary alcohol capping monomer **60** may be reacted with methane sulfonyl chloride to provide the secondary alcohol capping monomer mesylate **61**. The primary alcohol extension monomer **57** may be reacted with a capping reagent to provide a compound **62**. The capping reagent may be various capping reagents as described above. The compound **62** may be reacted with a B_2 de-blocking reagent to remove the blocking moiety B_2 and provide a secondary alcohol capping monomer **63**. The B_2 de-blocking reagent may be various de-blocking agents as will be understood by those skilled in the art including, but not limited to, H_2 in the presence of a palladium/activated carbon catalyst. The secondary alcohol capping monomer may be reacted with methanesulfonyl chloride to provide a primary alcohol capping monomer mesylate **64**. While the embodiments illustrated in **Figure 11** show the synthesis of capping monomers, it is to be understood that similar reactions may be performed to provide capping polymers.

In general, chain extensions may be effected by reacting a primary alcohol extension mono- or poly-mer such as the primary alcohol extension monomer **57** with a primary alcohol extension mono- or poly-mer mesylate such as the primary alcohol extension monomer mesylate **55** to provide various uniform polypropylene chains or by reacting a secondary alcohol extension mono- or poly-mer such as the secondary alcohol extension monomer **54** with a secondary alcohol extension mono-or poly-mer mesylate such as the secondary alcohol extension monomer mesylate **58**.

For example, in **Figure 13**, the primary alcohol extension monomer mesylate **55** is reacted with the primary alcohol extension monomer **57** to provide a dimer compound **65**. Alternatively, the secondary alcohol extension monomer mesylate **58** may be reacted with the secondary alcohol extension monomer **54** to provide the dimer compound **65**. The B_1 blocking moiety on the dimer compound **65** may be removed using a B_1 de-blocking reagent as described above to provide a primary alcohol extension dimer **66**. The primary alcohol extension dimer **66** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension dimer mesylate **67**. Alternatively, the B_2 blocking moiety on the dimer compound **65** may be removed using the B_2 de-blocking reagent as described above to provide a secondary alcohol extension dimer **69**. The secondary alcohol extension dimer **69** may be reacted with methane sulfonyl chloride to provide a primary alcohol extension dimer mesylate **70**.

As will be understood by those skilled in the art, the chain extension process may be repeated to achieve various other chain lengths. For example, as illustrated in **Figure 13**, the primary alcohol extension dimer **66** may be reacted with the primary alcohol extension dimer mesylate **70** to provide a tetramer compound **72**. As further illustrated in **Figure 13**, a generic chain extension reaction scheme involves reacting the primary alcohol extension mono- or poly-mer **73** with the primary alcohol extension mono- or poly-mer mesylate **74** to provide the uniform polypropylene polymer **75**. The values of m and n may each range from 0 to 1000 or more. Preferably, m and n are each from 0 to 50. While the embodiments illustrated in **Figure 13** show primary alcohol extension mono- and/or poly-mers being reacted with primary alcohol extension mono- and/or poly-mer mesylates, it is to be understood that similar reactions may be carried out using secondary alcohol extension mono- and/or poly-mers and secondary alcohol extension mono- and/or poly-mer mesylates.

An end of a primary alcohol extension mono- or poly-mer or an end of a primary alcohol extension mono- or poly-mer mesylate may be reacted with a primary alcohol capping mono- or poly-mer mesylate or a primary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the primary alcohol extension dimer mesylate **70** is reacted with the primary alcohol capping monomer **60** to provide the capped/blocked primary alcohol extension trimer **71**. As will be understood by those skilled in the art, the B_1 blocking moiety may be removed

and the resulting capped primary alcohol extension trimer may be reacted with a primary alcohol extension mono- or poly-mer mesylate to extend the chain of the capped trimer **71**.

An end of a secondary alcohol extension mono-or poly-mer or an end of a secondary alcohol extension mono-or poly-mer mesylate may be reacted with a secondary alcohol capping mono-or poly-mer mesylate or a secondary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the secondary alcohol extension dimer mesylate **67** is reacted with the secondary alcohol capping monomer **63** to provide the capped/blocked primary alcohol extension trimer **68**. The B_2 blocking moiety may be removed as described above and the resulting capped secondary alcohol extension trimer may be reacted with a secondary alcohol extension mer mesylate to extend the chain of the capped trimer **68**. While the syntheses illustrated in **Figure 12** show the reaction of a dimer with a capping monomer to provide a trimer, it is to be understood that the capping process may be performed at any point in the synthesis of a uniform polypropylene glycol moiety, or, alternatively, uniform polypropylene glycol moieties may be provided that are not capped. While the embodiments illustrated in **Figure 12** show the capping of a polybutylene oligomer by synthesis with a capping monomer, it is to be understood that polybutylene oligomers of the present invention may be capped directly (i.e., without the addition of a capping monomer) using a capping reagent as described above in **Figure 11**.

Uniform polypropylene glycol moieties according to embodiments of the present invention may be coupled to a growth hormone drug, a lipophilic moiety such as a carboxylic acid, and/or various other moieties by various methods as will be understood by those skilled in the art including, but not limited to, those described herein with respect to polyethylene glycol moieties.

The oligomer may comprise one or more other moieties as will be understood by those skilled in the art including, but not limited to, hydrophilic moieties, lipophilic moieties, spacer moieties, linker moieties, and terminating moieties. The various moieties in the oligomer are covalently coupled to one another by either hydrolyzable or non-hydrolyzable bonds.

The oligomer may further comprise one or more hydrophilic moieties including, but not limited to, sugars, polyalkylene glycols, and polyamine/PEG copolymers. Adjacent

polyalkylene glycol moieties will be considered to be the same moiety if they are coupled by an ether bond and have the same alkyl structure. For example, the moiety

$$—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—$$

is a single polyethylene glycol moiety having six polyethylene glycol subunits. Adjacent polyalkylene glycol moieties will be considered to be different moieties if they are coupled by a bond other than an ether bond or if they have different alkyl structures. For example, the moiety

$$—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—\overset{\overset{\displaystyle O}{\|}}{C}—O—C_2H_4—O—C_2H_4—$$

is a polyethylene glycol moiety having four polyethylene glycol subunits and a hydrophilic moiety having two polyethylene glycol subunits. Preferably, oligomers according to embodiments of the present invention comprise a polyalkylene glycol moiety and do not further comprise a hydrophilic moiety.

The oligomer may further comprise one or more lipophilic moieties as will be understood by those skilled in the art. The lipophilic moiety is preferably a saturated or unsaturated, linear or branched alkyl moiety or a saturated or unsaturated, linear or branched fatty acid moiety. When the lipophilic moiety is an alkyl moiety, it is preferably a linear, saturated or unsaturated alkyl moiety having 1 to 28 carbon atoms. More preferably, the alkyl moiety has 2 to 12 carbon atoms. When the lipophilic moiety is a fatty acid moiety, it is preferably a natural fatty acid moiety that is linear, saturated or unsaturated, having 2 to 18 carbon atoms. More preferably, the fatty acid moiety has 3 to 14 carbon atoms. Most preferably, the fatty acid moiety has at least 4, 5 or 6 carbon atoms.

The oligomer may further comprise one or more spacer moieties as will be understood by those skilled in the art. Spacer moieties may, for example, be used to separate a hydrophilic moiety from a lipophilic moiety, to separate a lipophilic moiety or hydrophilic moiety from the growth hormone drug, to separate a first hydrophilic or lipophilic moiety from a second hydrophilic or lipophilic moiety, or to separate a hydrophilic moiety or lipophilic moiety from a linker moiety. Spacer moieties are preferably selected from the group consisting of sugar, cholesterol and glycerine moieties.

The oligomer may further comprise one or more linker moieties that are used to couple the oligomer with the growth hormone drug as will be understood by those skilled in

the art. Linker moieties are preferably selected from the group consisting of alkyl and fatty acid moieties.

The oligomer may further comprise one or more terminating moieties at the one or more ends of the oligomer which are not coupled to the growth hormone drug. The terminating moiety is preferably an alkyl or alkoxy moiety, and is more preferably a lower alkyl or lower alkoxy moiety. Most preferably, the terminating moiety is methyl or methoxy. While the terminating moiety is preferably an alkyl or alkoxy moiety, it is to be understood that the terminating moiety may be various moieties as will be understood by those skilled in the art including, but not limited to, sugars, cholesterol, alcohols, and fatty acids.

The oligomer is preferably covalently coupled to the growth hormone drug. In some embodiments, the growth hormone drug is coupled to the oligomer utilizing a hydrolyzable bond (e.g., an ester or carbonate bond). A hydrolyzable coupling may provide a growth hormone drug-oligomer conjugate that acts as a prodrug. In certain instances, for example where the growth hormone drug-oligomer conjugate is inactive (i.e., the conjugate lacks the ability to affect the body through the growth hormone drug's primary mechanism of action), a hydrolyzable coupling may provide for a time-release or controlled-release effect, administering the growth hormone drug over a given time period as one or more oligomers are cleaved from their respective growth hormone drug-oligomer conjugates to provide the active drug. In other embodiments, the growth hormone drug is coupled to the oligomer utilizing a non-hydrolyzable bond (e.g., a carbamate, amide, or ether bond). Use of a non-hydrolyzable bond may be preferable when it is desirable to allow the growth hormone drug-oligomer conjugate to circulate in the bloodstream for an extended period of time, preferably at least 2 hours. When the oligomer is covalently coupled to the growth hormone drug, the oligomer further comprises one or more bonding moieties that are used to covalently couple the oligomer with the growth hormone drug as will be understood by those skilled in the art. Bonding moieties are preferably selected from the group consisting of covalent bond(s), ester moieties, carbonate moieties, carbamate moieties, amide moieties and secondary amine moieties. More than one moiety on the oligomer may be covalently coupled to the growth hormone drug.

While the oligomer is preferably covalently coupled to the growth hormone drug, it is to be understood that the oligomer may be non-covalently coupled to the growth hormone drug to form a non-covalently conjugated growth hormone drug-oligomer complex. As will

be understood by those skilled in the art, non-covalent couplings include, but are not limited to, hydrogen bonding, ionic bonding, Van der Waals bonding, and micellular or liposomal encapsulation. According to embodiments of the present invention, oligomers may be suitably constructed, modified and/or appropriately functionalized to impart the ability for non-covalent conjugation in a selected manner (e.g., to impart hydrogen bonding capability), as will be understood by those skilled in the art. According to other embodiments of present invention, oligomers may be derivatized with various compounds including, but not limited to, amino acids, oligopeptides, peptides, bile acids, bile acid derivatives, fatty acids, fatty acid derivatives, salicylic acids, salicylic acid derivatives, aminosalicylic acids, and aminosalicylic acid derivatives. The resulting oligomers can non-covalently couple (complex) with drug molecules, pharmaceutical products, and/or pharmaceutical excipients. The resulting complexes preferably have balanced lipophilic and hydrophilic properties. According to still other embodiments of the present invention, oligomers may be derivatized with amine and/or alkyl amines. Under suitable acidic conditions, the resulting oligomers can form non-covalently conjugated complexes with drug molecules, pharmaceutical products and/or pharmaceutical excipients. The products resulting from such complexation preferably have balanced lipophilic and hydrophilic properties.

More than one oligomer (i.e., a plurality of oligomers) may be coupled to the growth hormone drug. The oligomers in the plurality are preferably the same. However, it is to be understood that the oligomers in the plurality may be different from one another, or, alternatively, some of the oligomers in the plurality may be the same and some may be different. When a plurality of oligomers are coupled to the growth hormone drug, it may be preferable to couple one or more of the oligomers to the growth hormone drug with hydrolyzable bonds and couple one or more of the oligomers to the growth hormone drug with non-hydrolyzable bonds. Alternatively, all of the bonds coupling the plurality of oligomers to the growth hormone drug may be hydrolyzable, but have varying degrees of hydrolyzability such that, for example, one or more of the oligomers is rapidly removed from the growth hormone drug by hydrolysis in the body and one or more of the oligomers is slowly removed from the growth hormone drug by hydrolysis in the body.

The oligomer may be coupled to the growth hormone drug at various nucleophilic residues of the drug including, but not limited to, nucleophilic hydroxyl functions and/or amino functions. Nucleophilic hydroxyl functions may be found, for example, at serine

and/or tyrosine residues, and nucleophilic amino functions may be found, for example, at histidine and/or lysine residues, and/or at the one or more N-termini of the polypeptide. When an oligomer is coupled to the one or more N-termini of the growth hormone polypeptide, the coupling preferably forms a secondary amine. When the growth hormone drug is human growth hormone, for example, the oligomer may be coupled to an amino functionality of Phe^{1}, Lys^{38}, Lys^{41}, Lys^{70}, Lys^{115}, Lys^{140}, Lys^{145}, Lys^{158}, Lys^{168}, and/or Lys^{172}.

Substantially monodispersed mixtures of growth hormone drug-oligomer conjugates of the present invention may be synthesized by various methods. For example, a substantially monodispersed mixture of oligomers consisting of carboxylic acid and polyethylene glycol is synthesized by contacting a substantially monodispersed mixture of carboxylic acid with a substantially monodispersed mixture of polyethylene glycol under conditions sufficient to provide a substantially monodispersed mixture of oligomers. The oligomers of the substantially monodispersed mixture are then activated so that they are capable of reacting with a growth hormone drug to provide a growth hormone drug-oligomer conjugate. One embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 3** and described in Examples 11-18 hereinbelow. Another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 4** and described in Examples 19-24 hereinbelow. Still another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 5** and described in Examples 25-29 hereinbelow. Yet another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 6** and described in Examples 30-31 hereinbelow. Another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 7** and described in Examples 32-37 hereinbelow. Still another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 8** and described in Example 38 hereinbelow. Yet another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 9** and described in Example 39 hereinbelow. Another embodiment of a synthesis route for providing a substantially monodispersed mixture of activated oligomers is illustrated in **Figure 10** and described in Example 40 hereinbelow.

The substantially monodispersed mixture of activated oligomers may be reacted with a substantially monodispersed mixture of growth hormone drugs under conditions sufficient to provide a mixture of growth hormone drug-oligomer conjugates. Exemplary syntheses are described hereinbelow in Examples 40 through 42. As will be understood by those skilled in the art, the reaction conditions (e.g., selected molar ratios, solvent mixtures and/or pH) may be controlled such that the mixture of growth hormone drug-oligomer conjugates resulting from the reaction of the substantially monodispersed mixture of activated oligomers and the substantially monodispersed mixture of growth hormone drugs is a substantially monodispersed mixture. For example, conjugation at the amino functionality of lysine may be suppressed by maintaining the pH of the reaction solution below the pK_a of lysine. Alternatively, the mixture of growth hormone drug-oligomer conjugates may be separated and isolated utilizing, for example, HPLC to provide a substantially monodispersed mixture of growth hormone drug-oligomer conjugates, for example mono-, di-, or tri-conjugates. The degree of conjugation (e.g., whether the isolated molecule is a mono-, di-, or tri-conjugate) of a particular isolated conjugate may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, mass spectroscopy. The particular conjugate structure (e.g., whether the oligomer is at Phe^{1}, Lys^{38}, Lys^{41}, Lys^{70}, Lys^{115}, Lys^{140}, Lys^{145}, Lys^{158}, Lys^{168} or Lys^{172} of a human growth hormone monoconjugate) may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, sequence analysis, peptide mapping, selective enzymatic cleavage, and/or endopeptidase cleavage.

As will be understood by those skilled in the art, one or more of the reaction sites on the growth hormone drug may be blocked by, for example, reacting the growth hormone drug with a suitable blocking reagent such as N-tert-butoxycarbonyl (t-BOC), or N-(9-fluorenylmethoxycarbonyl) (N-FMOC). This process may be preferred, for example, when the growth hormone drug is a polypeptide and it is desired to form an unsaturated conjugate (i.e., a conjugate wherein not all nucleophilic residues are conjugated) having an oligomer at one or more of the N-termini of the polypeptide. Following such blocking, the substantially monodispersed mixture of blocked growth hormone drugs may be reacted with the substantially monodispersed mixture of activated oligomers to provide a mixture of growth hormone drug-oligomer conjugates having oligomer(s) coupled to one or more nucleophilic residues and having blocking moieties coupled to other nucleophilic residues. After the

conjugation reaction, the growth hormone drug-oligomer conjugates may be de-blocked as will be understood by those skilled in the art. If necessary, the mixture of growth hormone drug-oligomer conjugates may then be separated as described above to provide a substantially monodispersed mixture of growth hormone drug-oligomer conjugates. Alternatively, the mixture of growth hormone drug-oligomer conjugates may be separated prior to de-blocking.

Substantially monodispersed mixtures of growth hormone drug-oligomer conjugates according to embodiments of the present invention preferably have improved properties when compared with those of conventional mixtures. For example, a substantially monodispersed mixture of growth hormone drug-oligomer conjugates preferably has an *in vivo* activity that is greater than the *in vivo* activity of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture. As will be understood by those skilled in the art, the number average molecular weight of the substantially monodispersed mixture and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography such as gel permeation chromatography as described, for example, in H.R. Allcock & F.W. Lampe, CONTEMPORARY POLYMER CHEMISTRY 394-402 (2d. ed., 1991).

As another example, a substantially monodispersed mixture of growth hormone drug-oligomer conjugates preferably has an *in vitro* activity that is greater than the *in vitro* activity of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture. As will be understood by those skilled in the art, the number average molecular weight of the substantially monodispersed mixture and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography. The *in vitro* activity of a particular mixture may be measured by various methods, as will be understood by those skilled in the art. Preferably, the *in vitro* activity is measured using a Cytosensor® Microphysiometer commercially available from Molecular Devices Corporation of Sunnyvale, California. The microphysiometer monitors small changes in the rates of extracellular acidification in response to a drug being added to cultured cells in a Transwell® (Corning, Inc., Acton, Massachusetts). This response is proportional to the activity of the molecule under study.

As still another example, a substantially monodispersed mixture of growth hormone drug-oligomer conjugates preferably has an increased resistance to degradation by chymotrypsin when compared to the resistance to degradation by chymotrypsin of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture. As will be understood by those skilled in the art, the number average molecular weight of the substantially monodispersed mixture and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

As yet another example, a substantially monodispersed mixture of growth hormone drug-oligomer conjugates has an inter-subject variability that is less than the inter-subject variability of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the substantially monodispersed mixture. As will be understood by those skilled in the art, the number average molecular weight of the substantially monodispersed mixture and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography. The inter-subject variability may be measured by various methods as will be understood by those skilled in the art. The inter-subject variability is preferably calculated as follows. The area under a dose response curve (AUC) (i.e., the area between the dose-response curve and a baseline value) is determined for each subject. The average AUC for all subjects is determined by summing the AUCs of each subject and dividing the sum by the number of subjects. The absolute value of the difference between the subject's AUC and the average AUC is then determined for each subject. The absolute values of the differences obtained are then summed to give a value that represents the inter-subject variability. Lower values represent lower inter-subject variabilities and higher values represent higher inter-subject variabilities.

Substantially monodispersed mixtures of growth hormone drug-oligomer conjugates according to embodiments of the present invention preferably have two or more of the above-described improved properties. More preferably, substantially monodispersed mixtures of growth hormone drug-oligomer conjugates according to embodiments of the present invention have three or more of the above-described improved properties. Most preferably, substantially monodispersed mixtures of growth hormone drug-oligomer conjugates

according to embodiments of the present invention have all four of the above-described improved properties.

In still other embodiments according to the present invention, a mixture of conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons is provided. Each conjugate in the mixture includes a growth hormone drug coupled to an oligomer that comprises a polyalkylene glycol moiety. The standard deviation is preferably less than about 14 Daltons and is more preferably less than about 11 Daltons. The molecular weight distribution may be determined by methods known to those skilled in the art including, but not limited to, size exclusion chromatography such as gel permeation chromatography as described, for example, in H.R. Allcock & F.W. Lampe, CONTEMPORARY POLYMER CHEMISTRY 394-402 (2d. ed., 1991). The standard deviation of the molecular weight distribution may then be determined by statistical methods as will be understood by those skilled in the art.

The growth hormone drug is preferably human growth hormone. However, it is to be understood that the growth hormone drug may be selected from various growth hormone drugs known to those skilled in the art including, for example, growth hormone peptides, growth hormone peptide analogues, growth hormone peptide fragments, and growth hormone peptide fragment analogues. Growth hormone peptides include, but are not limited to, growth hormone, human (hGH); growth hormone, porcine; growth hormone, bovine; growth hormone, chicken; growth hormone, rat; growth hormone, mouse; growth hormone, ovine; growth hormone releasing factor, human; growth hormone pro-releasing factor, human; growth hormone releasing factor, mouse; growth hormone releasing factor, ovine; growth hormone releasing factor, rat; growth hormone releasing factor, bovine; growth hormone releasing factor, porcine; and growth hormone releasing factor, chicken. Growth hormone peptide analogs may be provided as described above by substituting one or more amino acids in a growth hormone peptide. Growth hormone peptide fragments include, but are not limited to, growth hormone 1-43, human; growth hormone 6-13; growth hormone releasing factor 1-37, human; growth hormone releasing factor 1-40, human; growth hormone releasing factor 1-40, amide, human; growth hormone releasing factor 30-44, amide, human; growth hormone releasing factor 1-29, amide, rat; hexarelin (growth hormone releasing hexapeptide); and growth hormone releasing factor 1-29, amide, human. Growth hormone peptide fragment analogues include, but are not limited to, [D-Ala^2]-growth hormone releasing factor

1-29, amide, human; [N-Ac-Tyr^{1}, D-Arg^{2}]-growth hormone releasing factor 1-29, amide; [His^{1}, Nle^{27}]-growth hormone releasing factor 1-32, amide; growth hormone releasing peptide-6 ([His^{1}, Lys^{6}]-GHRP); and [D-Lys^{3}]-GHRP-6.

The oligomer may be various oligomers comprising a polyalkylene glycol moiety as will be understood by those skilled in the art. Preferably, the polyalkylene glycol moiety has at least 2, 3, or 4 polyalkylene glycol subunits. More preferably, the polyalkylene glycol moiety has at least 5 or 6 polyalkylene glycol subunits. Most preferably, the polyalkylene glycol moiety of the oligomer has at least 7 polyalkylene glycol subunits. The polyalkylene glycol moiety of the oligomer is preferably a lower alkyl polyalkylene glycol moiety such as a polyethylene glycol moiety, a polypropylene glycol moiety, or a polybutylene glycol moiety. The polyalkylene glycol moiety is more preferably a polypropylene glycol moiety having a uniform structure. An exemplary polypropylene glycol moiety having a uniform structure is as follows:

$$-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-$$

This uniform polypropylene glycol structure may be described as having only one methyl substituted carbon atom adjacent each oxygen atom in the polypropylene glycol chain. Such uniform polypropylene glycol moieties may exhibit both lipophilic and hydrophilic characteristics and thus be useful in providing amphiphilic growth hormone drug-oligomer conjugates without the use of lipophilic polymer moieties. Furthermore, coupling the secondary alcohol moiety of the polypropylene glycol moiety with a growth hormone drug may provide the growth hormone drug (e.g., human growth hormone) with improved resistance to degradation caused by enzymes such as trypsin and chymotrypsin found, for example, in the gut.

Uniform polypropylene glycol according to embodiments of the present invention is preferably synthesized as illustrated in **Figures 11** through **13**, which will now be described. As illustrated in **Figure 11**, 1,2-propanediol **53** is reacted with a primary alcohol blocking reagent to provide a secondary alcohol extension monomer **54**. The primary alcohol blocking reagent may be various primary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, silylchloride compounds such as t-butyldiphenylsilylchloride and t-butyldimethylsilylchloride, and esterification reagents such as Ac_2O. Preferably, the primary alcohol blocking reagent is a primary alcohol blocking

reagent that is substantially non-reactive with secondary alcohols, such as t-butyldiphenylsilylchloride or t-butyldimethylsilylchloride. The secondary alcohol extension monomer (**54**) may be reacted with methanesulfonyl chloride ($MeSO_2Cl$) to provide a primary extension alcohol monomer mesylate **55**.

Alternatively, the secondary alcohol extension monomer **54** may be reacted with a secondary alcohol blocking reagent to provide compound **56**. The secondary alcohol blocking reagent may be various secondary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, benzyl chloride. The compound **56** may be reacted with a B_1 de-blocking reagent to remove the blocking moiety B_1 and provide a primary alcohol extension monomer **57**. The B_1 de-blocking reagent may be selected from various de-blocking reagents as will be understood by one skilled in the art. When the primary alcohol has been blocked by forming an ester, the B_1 de-blocking reagent is a de-esterification reagent, such as a base (e.g., potassium carbonate). When the primary alcohol has been blocked using a silylchloride, the B_1 de-blocking reagent is preferably tetrabutylammonium fluoride (TBAF). The primary alcohol extension monomer **57** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension monomer mesylate **58**.

The primary alcohol extension monomer **54** and the secondary alcohol extension monomer **57** may be capped as follows. The secondary alcohol extension monomer **54** may be reacted with a capping reagent to provide a compound **59**. The capping reagent may be various capping reagents as will be understood by those skilled in the art including, but not limited to, alkyl halides such as methyl chloride. The compound **59** may be reacted with a B_1 de-blocking agent as described above to provide a primary alcohol capping monomer **60**. The primary alcohol capping monomer **60** may be reacted with methane sulfonyl chloride to provide the secondary alcohol capping monomer mesylate **61**. The primary alcohol extension monomer **57** may be reacted with a capping reagent to provide a compound **62**. The capping reagent may be various capping reagents as described above. The compound **62** may be reacted with a B_2 de-blocking reagent to remove the blocking moiety B_2 and provide a secondary alcohol capping monomer **63**. The B_2 de-blocking reagent may be various de-blocking agents as will be understood by those skilled in the art including, but not limited to, H_2 in the presence of a palladium/activated carbon catalyst. The secondary alcohol capping monomer may be reacted with methanesulfonyl chloride to provide a primary alcohol

capping monomer mesylate **64**. While the embodiments illustrated in **Figure 11** show the synthesis of capping monomers, it is to be understood that similar reactions may be performed to provide capping polymers.

In general, chain extensions may be effected by reacting a primary alcohol extension mono- or poly-mer such as the primary alcohol extension monomer **57** with a primary alcohol extension mono- or poly-mer mesylate such as the primary alcohol extension monomer mesylate **55** to provide various uniform polypropylene chains or by reacting a secondary alcohol extension mono- or poly-mer such as the secondary alcohol extension monomer **54** with a secondary alcohol extension mono-or poly-mer mesylate such as the secondary alcohol extension monomer mesylate **58**.

For example, in **Figure 13**, the primary alcohol extension monomer mesylate **55** is reacted with the primary alcohol extension monomer **57** to provide a dimer compound **65**. Alternatively, the secondary alcohol extension monomer mesylate **58** may be reacted with the secondary alcohol extension monomer **54** to provide the dimer compound **65**. The B_1 blocking moiety on the dimer compound **65** may be removed using a B_1 de-blocking reagent as described above to provide a primary alcohol extension dimer **66**. The primary alcohol extension dimer **66** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension dimer mesylate **67**. Alternatively, the B_2 blocking moiety on the dimer compound **65** may be removed using the B_2 de-blocking reagent as described above to provide a secondary alcohol extension dimer **69**. The secondary alcohol extension dimer **69** may be reacted with methane sulfonyl chloride to provide a primary alcohol extension dimer mesylate **70**.

As will be understood by those skilled in the art, the chain extension process may be repeated to achieve various other chain lengths. For example, as illustrated in **Figure 13**, the primary alcohol extension dimer **66** may be reacted with the primary alcohol extension dimer mesylate **70** to provide a tetramer compound **72**. As further illustrated in **Figure 13**, a generic chain extension reaction scheme involves reacting the primary alcohol extension mono- or poly-mer **73** with the primary alcohol extension mono- or poly-mer mesylate **74** to provide the uniform polypropylene polymer **75**. The values of m and n may each range from 0 to 1000 or more. Preferably, m and n are each from 0 to 50. While the embodiments illustrated in **Figure 13** show primary alcohol extension mono- and/or poly-mers being reacted with primary alcohol extension mono- and/or poly-mer mesylates, it is to be

understood that similar reactions may be carried out using secondary alcohol extension mono- and/or poly-mers and secondary alcohol extension mono- and/or poly-mer mesylates.

An end of a primary alcohol extension mono- or poly-mer or an end of a primary alcohol extension mono- or poly-mer mesylate may be reacted with a primary alcohol capping mono- or poly-mer mesylate or a primary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the primary alcohol extension dimer mesylate **70** is reacted with the primary alcohol capping monomer **60** to provide the capped/blocked primary alcohol extension trimer **71**. As will be understood by those skilled in the art, the B_1 blocking moiety may be removed and the resulting capped primary alcohol extension trimer may be reacted with a primary alcohol extension mono- or poly-mer mesylate to extend the chain of the capped trimer **71**.

An end of a secondary alcohol extension mono-or poly-mer or an end of a secondary alcohol extension mono-or poly-mer mesylate may be reacted with a secondary alcohol capping mono-or poly-mer mesylate or a secondary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the secondary alcohol extension dimer mesylate **67** is reacted with the secondary alcohol capping monomer **63** to provide the capped/blocked primary alcohol extension trimer **68**. The B_2 blocking moiety may be removed as described above and the resulting capped secondary alcohol extension trimer may be reacted with a secondary alcohol extension mer mesylate to extend the chain of the capped trimer **68**. While the syntheses illustrated in **Figure 12** show the reaction of a dimer with a capping monomer to provide a trimer, it is to be understood that the capping process may be performed at any point in the synthesis of a uniform polypropylene glycol moiety, or, alternatively, uniform polypropylene glycol moieties may be provided that are not capped. While the embodiments illustrated in **Figure 12** show the capping of a polybutylene oligomer by synthesis with a capping monomer, it is to be understood that polybutylene oligomers of the present invention may be capped directly (i.e., without the addition of a capping monomer) using a capping reagent as described above in **Figure 11**.

Uniform polypropylene glycol moieties according to embodiments of the present invention may be coupled to a growth hormone drug, a lipophilic moiety such as a carboxylic acid, and/or various other moieties by various methods as will be understood by those skilled

in the art including, but not limited to, those described herein with respect to polyethylene glycol moieties.

The oligomer may comprise one or more other moieties as will be understood by those skilled in the art including, but not limited to, hydrophilic moieties, lipophilic moieties, spacer moieties, linker moieties, and terminating moieties. The various moieties in the oligomer are covalently coupled to one another by either hydrolyzable or non-hydrolyzable bonds.

The oligomer may further comprise one or more hydrophilic moieties including, but not limited to, sugars, polyalkylene glycols, and polyamine/PEG copolymers. Adjacent polyalkylene glycol moieties will be considered to be the same moiety if they are coupled by an ether bond and have the same alkyl structure. For example, the moiety

$$—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—$$

is a single polyethylene glycol moiety having six polyethylene glycol subunits. Adjacent polyalkylene glycol moieties will be considered to be different moieties if they are coupled by a bond other than an ether bond or if they have different alkyl structures. For example, the moiety

$$—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—\overset{\overset{\displaystyle O}{\|}}{C}—O—C_2H_4—O—C_2H_4—$$

is a polyethylene glycol moiety having four polyethylene glycol subunits and a hydrophilic moiety having two polyethylene glycol subunits. Preferably, oligomers according to embodiments of the present invention comprise a polyalkylene glycol moiety and do not further comprise a hydrophilic moiety.

The oligomer may further comprise one or more lipophilic moieties as will be understood by those skilled in the art. The lipophilic moiety is preferably a saturated or unsaturated, linear or branched alkyl moiety or a saturated or unsaturated, linear or branched fatty acid moiety. When the lipophilic moiety is an alkyl moiety, it is preferably a linear, saturated or unsaturated alkyl moiety having 1 to 28 carbon atoms. More preferably, the alkyl moiety has 2 to 12 carbon atoms. When the lipophilic moiety is a fatty acid moiety, it is preferably a natural fatty acid moiety that is linear, saturated or unsaturated, having 2 to 18 carbon atoms. More preferably, the fatty acid moiety has 3 to 14 carbon atoms. Most preferably, the fatty acid moiety has at least 4, 5 or 6 carbon atoms.

The oligomer may further comprise one or more spacer moieties as will be understood by those skilled in the art. Spacer moieties may, for example, be used to separate a hydrophilic moiety from a lipophilic moiety, to separate a lipophilic moiety or hydrophilic moiety from the growth hormone drug, to separate a first hydrophilic or lipophilic moiety from a second hydrophilic or lipophilic moiety, or to separate a hydrophilic moiety or lipophilic moiety from a linker moiety. Spacer moieties are preferably selected from the group consisting of sugar, cholesterol and glycerine moieties.

The oligomer may further comprise one or more linker moieties that are used to couple the oligomer with the growth hormone drug as will be understood by those skilled in the art. Linker moieties are preferably selected from the group consisting of alkyl and fatty acid moieties.

The oligomer may further comprise one or more terminating moieties at the one or more ends of the oligomer which are not coupled to the growth hormone drug. The terminating moiety is preferably an alkyl or alkoxy moiety, and is more preferably a lower alkyl or lower alkoxy moiety. Most preferably, the terminating moiety is methyl or methoxy. While the terminating moiety is preferably an alkyl or alkoxy moiety, it is to be understood that the terminating moiety may be various moieties as will be understood by those skilled in the art including, but not limited to, sugars, cholesterol, alcohols, and fatty acids.

The oligomer is preferably covalently coupled to the growth hormone drug. In some embodiments, the growth hormone drug is coupled to the oligomer utilizing a hydrolyzable bond (e.g., an ester or carbonate bond). A hydrolyzable coupling may provide a growth hormone drug-oligomer conjugate that acts as a prodrug. In certain instances, for example where the growth hormone drug-oligomer conjugate is inactive (i.e., the conjugate lacks the ability to affect the body through the growth hormone drug's primary mechanism of action), a hydrolyzable coupling may provide for a time-release or controlled-release effect, administering the growth hormone drug over a given time period as one or more oligomers are cleaved from their respective growth hormone drug-oligomer conjugates to provide the active drug. In other embodiments, the growth hormone drug is coupled to the oligomer utilizing a non-hydrolyzable bond (e.g., a carbamate, amide, or ether bond). Use of a non-hydrolyzable bond may be preferable when it is desirable to allow the growth hormone drug-oligomer conjugate to circulate in the bloodstream for an extended period of time, preferably at least 2 hours. When the oligomer is covalently coupled to the growth hormone drug, the

oligomer further comprises one or more bonding moieties that are used to covalently couple the oligomer with the growth hormone drug as will be understood by those skilled in the art. Bonding moieties are preferably selected from the group consisting of covalent bond(s), ester moieties, carbonate moieties, carbamate moieties, amide moieties and secondary amine moieties. More than one moiety on the oligomer may be covalently coupled to the growth hormone drug.

While the oligomer is preferably covalently coupled to the growth hormone drug, it is to be understood that the oligomer may be non-covalently coupled to the growth hormone drug to form a non-covalently conjugated growth hormone drug-oligomer complex. As will be understood by those skilled in the art, non-covalent couplings include, but are not limited to, hydrogen bonding, ionic bonding, Van der Waals bonding, and micellular or liposomal encapsulation. According to embodiments of the present invention, oligomers may be suitably constructed, modified and/or appropriately functionalized to impart the ability for non-covalent conjugation in a selected manner (e.g., to impart hydrogen bonding capability), as will be understood by those skilled in the art. According to other embodiments of present invention, oligomers may be derivatized with various compounds including, but not limited to, amino acids, oligopeptides, peptides, bile acids, bile acid derivatives, fatty acids, fatty acid derivatives, salicylic acids, salicylic acid derivatives, aminosalicylic acids, and aminosalicylic acid derivatives. The resulting oligomers can non-covalently couple (complex) with drug molecules, pharmaceutical products, and/or pharmaceutical excipients. The resulting complexes preferably have balanced lipophilic and hydrophilic properties. According to still other embodiments of the present invention, oligomers may be derivatized with amine and/or alkyl amines. Under suitable acidic conditions, the resulting oligomers can form non-covalently conjugated complexes with drug molecules, pharmaceutical products and/or pharmaceutical excipients. The products resulting from such complexation preferably have balanced lipophilic and hydrophilic properties.

More than one oligomer (i.e., a plurality of oligomers) may be coupled to the growth hormone drug. The oligomers in the plurality are preferably the same. However, it is to be understood that the oligomers in the plurality may be different from one another, or, alternatively, some of the oligomers in the plurality may be the same and some may be different. When a plurality of oligomers are coupled to the growth hormone drug, it may be preferable to couple one or more of the oligomers to the growth hormone drug with

hydrolyzable bonds and couple one or more of the oligomers to the growth hormone drug with non-hydrolyzable bonds. Alternatively, all of the bonds coupling the plurality of oligomers to the growth hormone drug may be hydrolyzable, but have varying degrees of hydrolyzability such that, for example, one or more of the oligomers is rapidly removed from the growth hormone drug by hydrolysis in the body and one or more of the oligomers is slowly removed from the growth hormone drug by hydrolysis in the body.

The oligomer may be coupled to the growth hormone drug at various nucleophilic residues of the drug including, but not limited to, nucleophilic hydroxyl functions and/or amino functions. Nucleophilic hydroxyl functions may be found, for example, at serine and/or tyrosine residues, and nucleophilic amino functions may be found, for example, at histidine and/or lysine residues, and/or at the one or more N-termini of the polypeptide. When an oligomer is coupled to the one or more N-termini of the growth hormone polypeptide, the coupling preferably forms a secondary amine. When the growth hormone drug is human growth hormone, for example, the oligomer may be coupled to an amino functionality of Phe^{1}, Lys^{38}, Lys^{41}, Lys^{70}, Lys^{115}, Lys^{140}, Lys^{145}, Lys^{158}, Lys^{168}, and/or Lys^{172}.

Mixtures of growth hormone drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons may be synthesized by various methods. For example, a mixture of oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons consisting of carboxylic acid and polyethylene glycol is synthesized by contacting a mixture of carboxylic acid having a molecular weight distribution with a standard deviation of less than about 22 Daltons with a mixture of polyethylene glycol having a molecular weight distribution with a standard deviation of less than about 22 Daltons under conditions sufficient to provide a mixture of oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons. The oligomers of the mixture having a molecular weight distribution with a standard deviation of less than about 22 Daltons are then activated so that they are capable of reacting with a growth hormone drug to provide a growth hormone drug-oligomer conjugate. One embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 3** and described in Examples 11-18 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 4**

and described in Examples 19-24 hereinbelow. Still another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 5** and described in Examples 25-29 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 6** and described in Examples 30-31 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 7** and described in Examples 32-37 hereinbelow. Still another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 8** and described in Example 38 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 9** and described in Example 39 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is illustrated in **Figure 10** and described in Example 40 hereinbelow.

The mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons is reacted with a mixture of growth hormone drugs having a molecular weight distribution with a standard deviation of less than about 22 Daltons under conditions sufficient to provide a mixture of growth hormone drug-oligomer conjugates. Exemplary syntheses are described hereinbelow in Examples 40 through 42. As will be understood by those skilled in the art, the reaction conditions (e.g., selected molar ratios, solvent mixtures and/or pH) may be controlled such that the mixture of growth hormone drug-oligomer conjugates resulting from the reaction of the mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons and the mixture of growth hormone drugs having a molecular weight distribution with a standard deviation of less than about 22 Daltons is a mixture having a molecular weight distribution with a standard deviation of less than about 22 Daltons. For example, conjugation at the amino functionality of lysine may be suppressed by maintaining the pH of the reaction solution below the pK_a of lysine. Alternatively, the mixture of growth hormone

drug-oligomer conjugates may be separated and isolated utilizing, for example, HPLC to provide a mixture of growth hormone drug-oligomer conjugates, for example mono-, di-, or tri-conjugates, having a molecular weight distribution with a standard deviation of less than about 22 Daltons. The degree of conjugation (e.g., whether the isolated molecule is a mono-, di-, or tri-conjugate) of a particular isolated conjugate may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, mass spectroscopy. The particular conjugate structure (e.g., whether the oligomer is at Phe^{1}, Lys^{38}, Lys^{41}, Lys^{70}, Lys^{115}, Lys^{140}, Lys^{145}, Lys^{158}, Lys^{168} or Lys^{172} of a human growth hormone monoconjugate) may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, sequence analysis, peptide mapping, selective enzymatic cleavage, and/or endopeptidase cleavage.

As will be understood by those skilled in the art, one or more of the reaction sites on the growth hormone drug may be blocked by, for example, reacting the growth hormone drug with a suitable blocking reagent such as N-tert-butoxycarbonyl (t-BOC), or N-(9-fluorenylmethoxycarbonyl) (N-FMOC). This process may be preferred, for example, when the growth hormone drug is a polypeptide and it is desired to form an unsaturated conjugate (i.e., a conjugate wherein not all nucleophilic residues are conjugated) having one or more oligomers at the one or more N-termini of the polypeptide. Following such blocking, the mixture of blocked growth hormone drugs having a molecular weight distribution with a standard deviation of less than about 22 Daltons may be reacted with the mixture of activated oligomers having a molecular weight distribution with a standard deviation of less than about 22 Daltons to provide a mixture of growth hormone drug-oligomer conjugates having oligomer(s) coupled to one or more nucleophilic residues and having blocking moieties coupled to other nucleophilic residues. After the conjugation reaction, the growth hormone drug-oligomer conjugates may be de-blocked as will be understood by those skilled in the art. If necessary, the mixture of growth hormone drug-oligomer conjugates may then be separated as described above to provide a mixture of growth hormone drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons. Alternatively, the mixture of growth hormone drug-oligomer conjugates may be separated prior to de-blocking.

Mixtures of growth hormone drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons according to embodiments of the present invention preferably have improved properties when compared with those of conventional mixtures. For example, a mixture of growth hormone drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons preferably has an *in vivo* activity that is greater than the *in vivo* activity of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the mixture of growth hormone drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons. As will be understood by those skilled in the art, the number average molecular weight of the mixture of growth hormone drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography such as gel permeation chromatography as described, for example, in H.R. Allcock & F.W. Lampe, CONTEMPORARY POLYMER CHEMISTRY 394-402 (2d. ed., 1991).

As another example, a mixture of growth hormone drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons preferably has an *in vitro* activity that is greater than the *in vitro* activity of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the mixture of growth hormone drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons. As will be understood by those skilled in the art, the number average molecular weight of the mixture of growth hormone drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

The *in vitro* activity of a particular mixture may be measured by various methods, as will be understood by those skilled in the art. Preferably, the *in vitro* activity is measured using a Cytosensor® Microphysiometer commercially available from Molecular Devices Corporation of Sunnyvale, California. The microphysiometer monitors small changes in the

rates of extracellular acidification in response to a drug being added to cultured cells in a transwell. This response is proportional to the activity of the molecule under study.

As still another example, a mixture of growth hormone drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons preferably has an increased resistance to degradation by chymotrypsin when compared to the resistance to degradation by chymotrypsin of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the mixture of growth hormone drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons. As will be understood by those skilled in the art, the number average molecular weight of the mixture of growth hormone drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

As yet another example, a mixture of growth hormone drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons preferably has an inter-subject variability that is less than the inter-subject variability of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the mixture of growth hormone drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons. As will be understood by those skilled in the art, the number average molecular weight of the mixture of growth hormone drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography. The inter-subject variability may be measured by various methods as will be understood by those skilled in the art. The inter-subject variability is preferably calculated as follows. The area under a dose response curve (AUC) (i.e., the area between the dose-response curve and a baseline value) is determined for each subject. The average AUC for all subjects is determined by summing the AUCs of each subject and dividing the sum by the number of subjects. The absolute value of the difference between the subject's AUC and the average AUC is then determined for each subject. The absolute values of the differences obtained are then summed to give a value that

represents the inter-subject variability. Lower values represent lower inter-subject variabilities and higher values represent higher inter-subject variabilities.

Mixtures of growth hormone drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons according to embodiments of the present invention preferably have two or more of the above-described improved properties. More preferably, mixtures of growth hormone drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons according to embodiments of the present invention have three or more of the above-described improved properties. Most preferably, mixtures of growth hormone drug-oligomer conjugates having a molecular weight distribution with a standard deviation of less than about 22 Daltons according to embodiments of the present invention have all four of the above-described improved properties.

According to yet other embodiments of the present invention, a mixture of conjugates is provided where each conjugate includes a growth hormone drug coupled to an oligomer that comprises a polyalkylene glycol moiety, and the mixture has a dispersity coefficient (DC) greater than 10,000 where

$$DC = \frac{\left(\sum_{i=1}^{n} N_i M_i\right)^2}{\sum_{i=1}^{n} N_i M_i^2 \sum_{i=1}^{n} N_i - \left(\sum_{i=1}^{n} N_i M_i\right)^2}$$

wherein:

n is the number of different molecules in the sample;

N_i is the number of $i^{\underline{th}}$ molecules in the sample; and

M_i is the mass of the $i^{\underline{th}}$ molecule.

The mixture of conjugates preferably has a dispersity coefficient greater than 100,000. More preferably, the dispersity coefficient of the conjugate mixture is greater than 500,000 and, most preferably, the dispersity coefficient is greater than 10,000,000. The variables n, N_i, and M_i may be determined by various methods as will be understood by those skilled in the art, including, but not limited to, methods described below in Example 44.

The growth hormone drug is preferably human growth hormone. However, it is to be understood that the growth hormone drug may be selected from various growth hormone drugs known to those skilled in the art including, for example, growth hormone peptides,

growth hormone peptide analogues, growth hormone peptide fragments, and growth hormone peptide fragment analogues. Growth hormone peptides include, but are not limited to, growth hormone, human (hGH); growth hormone, porcine; growth hormone, bovine; growth hormone, chicken; growth hormone, rat; growth hormone, mouse; growth hormone, ovine; growth hormone releasing factor, human; growth hormone pro-releasing factor, human; growth hormone releasing factor, mouse; growth hormone releasing factor, ovine; growth hormone releasing factor, rat; growth hormone releasing factor, bovine; growth hormone releasing factor, porcine; and growth hormone releasing factor, chicken. Growth hormone peptide analogs may be provided as described above by substituting one or more amino acids in a growth hormone peptide. Growth hormone peptide fragments include, but are not limited to, growth hormone 1-43, human; growth hormone 6-13; growth hormone releasing factor 1-37, human; growth hormone releasing factor 1-40, human; growth hormone releasing factor 1-40, amide, human; growth hormone releasing factor 30-44, amide, human; growth hormone releasing factor 1-29, amide, rat; hexarelin (growth hormone releasing hexapeptide); and growth hormone releasing factor 1-29, amide, human. Growth hormone peptide fragment analogues include, but are not limited to, [D-Ala^{2}]-growth hormone releasing factor 1-29, amide, human; [N-Ac-Tyr^{1}, D-Arg^{2}]-growth hormone releasing factor 1-29, amide; [His^{1}, Nle^{27}]-growth hormone releasing factor 1-32, amide; growth hormone releasing peptide-6 ([His^{1}, Lys^{6}]-GHRP); and [D-Lys^{3}]-GHRP-6.

The oligomer may be various oligomers comprising a polyalkylene glycol moiety as will be understood by those skilled in the art. Preferably, the polyalkylene glycol moiety has at least 2, 3, or 4 polyalkylene glycol subunits. More preferably, the polyalkylene glycol moiety has at least 5 or 6 polyalkylene glycol subunits. Most preferably, the polyalkylene glycol moiety of the oligomer has at least 7 polyalkylene glycol subunits. The polyalkylene glycol moiety of the oligomer is preferably a lower alkyl polyalkylene glycol moiety such as a polyethylene glycol moiety, a polypropylene glycol moiety, or a polybutylene glycol moiety. The polyalkylene glycol moiety is more preferably a polypropylene glycol moiety having a uniform structure. An exemplary polypropylene glycol moiety having a uniform structure is as follows:

$$-O-\underset{CH_3}{\underset{|}{CH}}-CH_2-O-\underset{CH_3}{\underset{|}{CH}}-CH_2-O-\underset{CH_3}{\underset{|}{CH}}-CH_2-O-\underset{CH_3}{\underset{|}{CH}}-CH_2-O-$$

This uniform polypropylene glycol structure may be described as having only one methyl substituted carbon atom adjacent each oxygen atom in the polypropylene glycol chain. Such uniform polypropylene glycol moieties may exhibit both lipophilic and hydrophilic characteristics and thus be useful in providing amphiphilic growth hormone drug-oligomer conjugates without the use of lipophilic polymer moieties. Furthermore, coupling the secondary alcohol moiety of the polypropylene glycol moiety with a growth hormone drug may provide the growth hormone drug (e.g., human growth hormone) with improved resistance to degradation caused by enzymes such as trypsin and chymotrypsin found, for example, in the gut.

Uniform polypropylene glycol according to embodiments of the present invention is preferably synthesized as illustrated in **Figures 11** through **13**, which will now be described. As illustrated in **Figure 11**, 1,2-propanediol **53** is reacted with a primary alcohol blocking reagent to provide a secondary alcohol extension monomer **54**. The primary alcohol blocking reagent may be various primary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, silylchloride compounds such as t-butyldiphenylsilylchloride and t-butyldimethylsilylchloride, and esterification reagents such as Ac_2O. Preferably, the primary alcohol blocking reagent is a primary alcohol blocking reagent that is substantially non-reactive with secondary alcohols, such as t-butyldiphenylsilylchloride or t-butyldimethylsilylchloride. The secondary alcohol extension monomer (**54**) may be reacted with methanesulfonyl chloride ($MeSO_2Cl$) to provide a primary extension alcohol monomer mesylate **55**.

Alternatively, the secondary alcohol extension monomer **54** may be reacted with a secondary alcohol blocking reagent to provide compound **56**. The secondary alcohol blocking reagent may be various secondary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, benzyl chloride. The compound **56** may be reacted with a B_1 de-blocking reagent to remove the blocking moiety B_1 and provide a primary alcohol extension monomer **57**. The B_1 de-blocking reagent may be selected from various de-blocking reagents as will be understood by one skilled in the art. When the primary alcohol has been blocked by forming an ester, the B_1 de-blocking reagent is a de-esterification reagent, such as a base (e.g., potassium carbonate). When the primary alcohol has been blocked using a silylchloride, the B_1 de-blocking reagent is preferably tetrabutylammonium fluoride (TBAF). The primary alcohol extension monomer **57** may be

reacted with methane sulfonyl chloride to provide a secondary alcohol extension monomer mesylate **58**.

The primary alcohol extension monomer **54** and the secondary alcohol extension monomer **57** may be capped as follows. The secondary alcohol extension monomer **54** may be reacted with a capping reagent to provide a compound **59**. The capping reagent may be various capping reagents as will be understood by those skilled in the art including, but not limited to, alkyl halides such as methyl chloride. The compound **59** may be reacted with a B_1 de-blocking agent as described above to provide a primary alcohol capping monomer **60**. The primary alcohol capping monomer **60** may be reacted with methane sulfonyl chloride to provide the secondary alcohol capping monomer mesylate **61**. The primary alcohol extension monomer **57** may be reacted with a capping reagent to provide a compound **62**. The capping reagent may be various capping reagents as described above. The compound **62** may be reacted with a B_2 de-blocking reagent to remove the blocking moiety B_2 and provide a secondary alcohol capping monomer **63**. The B_2 de-blocking reagent may be various de-blocking agents as will be understood by those skilled in the art including, but not limited to, H_2 in the presence of a palladium/activated carbon catalyst. The secondary alcohol capping monomer may be reacted with methanesulfonyl chloride to provide a primary alcohol capping monomer mesylate **64**. While the embodiments illustrated in **Figure 11** show the synthesis of capping monomers, it is to be understood that similar reactions may be performed to provide capping polymers.

In general, chain extensions may be effected by reacting a primary alcohol extension mono- or poly-mer such as the primary alcohol extension monomer **57** with a primary alcohol extension mono- or poly-mer mesylate such as the primary alcohol extension monomer mesylate **55** to provide various uniform polypropylene chains or by reacting a secondary alcohol extension mono- or poly-mer such as the secondary alcohol extension monomer **54** with a secondary alcohol extension mono-or poly-mer mesylate such as the secondary alcohol extension monomer mesylate **58**.

For example, in **Figure 13**, the primary alcohol extension monomer mesylate **55** is reacted with the primary alcohol extension monomer **57** to provide a dimer compound **65**. Alternatively, the secondary alcohol extension monomer mesylate **58** may be reacted with the secondary alcohol extension monomer **54** to provide the dimer compound **65**. The B_1 blocking moiety on the dimer compound **65** may be removed using a B_1 de-blocking reagent

as described above to provide a primary alcohol extension dimer **66**. The primary alcohol extension dimer **66** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension dimer mesylate **67**. Alternatively, the B_2 blocking moiety on the dimer compound **65** may be removed using the B_2 de-blocking reagent as described above to provide a secondary alcohol extension dimer **69**. The secondary alcohol extension dimer **69** may be reacted with methane sulfonyl chloride to provide a primary alcohol extension dimer mesylate **70**.

As will be understood by those skilled in the art, the chain extension process may be repeated to achieve various other chain lengths. For example, as illustrated in **Figure 13**, the primary alcohol extension dimer **66** may be reacted with the primary alcohol extension dimer mesylate **70** to provide a tetramer compound **72**. As further illustrated in **Figure 13**, a generic chain extension reaction scheme involves reacting the primary alcohol extension mono- or poly-mer **73** with the primary alcohol extension mono- or poly-mer mesylate **74** to provide the uniform polypropylene polymer **75**. The values of m and n may each range from 0 to 1000 or more. Preferably, m and n are each from 0 to 50. While the embodiments illustrated in **Figure 13** show primary alcohol extension mono- and/or poly-mers being reacted with primary alcohol extension mono- and/or poly-mer mesylates, it is to be understood that similar reactions may be carried out using secondary alcohol extension mono- and/or poly-mers and secondary alcohol extension mono- and/or poly-mer mesylates.

An end of a primary alcohol extension mono- or poly-mer or an end of a primary alcohol extension mono- or poly-mer mesylate may be reacted with a primary alcohol capping mono- or poly-mer mesylate or a primary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the primary alcohol extension dimer mesylate **70** is reacted with the primary alcohol capping monomer **60** to provide the capped/blocked primary alcohol extension trimer **71**. As will be understood by those skilled in the art, the B_1 blocking moiety may be removed and the resulting capped primary alcohol extension trimer may be reacted with a primary alcohol extension mono- or poly-mer mesylate to extend the chain of the capped trimer **71**.

An end of a secondary alcohol extension mono-or poly-mer or an end of a secondary alcohol extension mono-or poly-mer mesylate may be reacted with a secondary alcohol capping mono-or poly-mer mesylate or a secondary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in

Figure 12, the secondary alcohol extension dimer mesylate **67** is reacted with the secondary alcohol capping monomer **63** to provide the capped/blocked primary alcohol extension trimer **68**. The B_2 blocking moiety may be removed as described above and the resulting capped secondary alcohol extension trimer may be reacted with a secondary alcohol extension mer mesylate to extend the chain of the capped trimer **68**. While the syntheses illustrated in **Figure 12** show the reaction of a dimer with a capping monomer to provide a trimer, it is to be understood that the capping process may be performed at any point in the synthesis of a uniform polypropylene glycol moiety, or, alternatively, uniform polypropylene glycol moieties may be provided that are not capped. While the embodiments illustrated in **Figure 12** show the capping of a polybutylene oligomer by synthesis with a capping monomer, it is to be understood that polybutylene oligomers of the present invention may be capped directly (i.e., without the addition of a capping monomer) using a capping reagent as described above in **Figure 11**.

Uniform polypropylene glycol moieties according to embodiments of the present invention may be coupled to a growth hormone drug, a lipophilic moiety such as a carboxylic acid, and/or various other moieties by various methods as will be understood by those skilled in the art including, but not limited to, those described herein with respect to polyethylene glycol moieties.

The oligomer may comprise one or more other moieties as will be understood by those skilled in the art including, but not limited to, hydrophilic moieties, lipophilic moieties, spacer moieties, linker moieties, and terminating moieties. The various moieties in the oligomer are covalently coupled to one another by either hydrolyzable or non-hydrolyzable bonds.

The oligomer may further comprise one or more hydrophilic moieties including, but not limited to, sugars, polyalkylene glycols, and polyamine/PEG copolymers. Adjacent polyalkylene glycol moieties will be considered to be the same moiety if they are coupled by an ether bond and have the same alkyl structure. For example, the moiety

$$—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—$$

is a single polyethylene glycol moiety having six polyethylene glycol subunits. Adjacent polyalkylene glycol moieties will be considered to be different moieties if they are coupled by a bond other than an ether bond or if they have different alkyl structures. For example, the moiety

$$—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—\overset{\overset{\displaystyle O}{\|}}{C}—O—C_2H_4—O—C_2H_4—$$

is a polyethylene glycol moiety having four polyethylene glycol subunits and a hydrophilic moiety having two polyethylene glycol subunits. Preferably, oligomers according to embodiments of the present invention comprise a polyalkylene glycol moiety and do not further comprise a hydrophilic moiety.

The oligomer may further comprise one or more lipophilic moieties as will be understood by those skilled in the art. The lipophilic moiety is preferably a saturated or unsaturated, linear or branched alkyl moiety or a saturated or unsaturated, linear or branched fatty acid moiety. When the lipophilic moiety is an alkyl moiety, it is preferably a linear, saturated or unsaturated alkyl moiety having 1 to 28 carbon atoms. More preferably, the alkyl moiety has 2 to 12 carbon atoms. When the lipophilic moiety is a fatty acid moiety, it is preferably a natural fatty acid moiety that is linear, saturated or unsaturated, having 2 to 18 carbon atoms. More preferably, the fatty acid moiety has 3 to 14 carbon atoms. Most preferably, the fatty acid moiety has at least 4, 5 or 6 carbon atoms.

The oligomer may further comprise one or more spacer moieties as will be understood by those skilled in the art. Spacer moieties may, for example, be used to separate a hydrophilic moiety from a lipophilic moiety, to separate a lipophilic moiety or hydrophilic moiety from the growth hormone drug, to separate a first hydrophilic or lipophilic moiety from a second hydrophilic or lipophilic moiety, or to separate a hydrophilic moiety or lipophilic moiety from a linker moiety. Spacer moieties are preferably selected from the group consisting of sugar, cholesterol and glycerine moieties.

The oligomer may further comprise one or more linker moieties that are used to couple the oligomer with the growth hormone drug as will be understood by those skilled in the art. Linker moieties are preferably selected from the group consisting of alkyl and fatty acid moieties.

The oligomer may further comprise one or more terminating moieties at the one or more ends of the oligomer which are not coupled to the growth hormone drug. The terminating moiety is preferably an alkyl or alkoxy moiety, and is more preferably a lower alkyl or lower alkoxy moiety. Most preferably, the terminating moiety is methyl or methoxy. While the terminating moiety is preferably an alkyl or alkoxy moiety, it is to be understood

that the terminating moiety may be various moieties as will be understood by those skilled in the art including, but not limited to, sugars, cholesterol, alcohols, and fatty acids.

The oligomer is preferably covalently coupled to the growth hormone drug. In some embodiments, the growth hormone drug is coupled to the oligomer utilizing a hydrolyzable bond (e.g., an ester or carbonate bond). A hydrolyzable coupling may provide a growth hormone drug-oligomer conjugate that acts as a prodrug. In certain instances, for example where the growth hormone drug-oligomer conjugate is inactive (i.e., the conjugate lacks the ability to affect the body through the growth hormone drug's primary mechanism of action), a hydrolyzable coupling may provide for a time-release or controlled-release effect, administering the growth hormone drug over a given time period as one or more oligomers are cleaved from their respective growth hormone drug-oligomer conjugates to provide the active drug. In other embodiments, the growth hormone drug is coupled to the oligomer utilizing a non-hydrolyzable bond (e.g., a carbamate, amide, or ether bond). Use of a non-hydrolyzable bond may be preferable when it is desirable to allow the growth hormone drug-oligomer conjugate to circulate in the bloodstream for an extended period of time, preferably at least 2 hours. When the oligomer is covalently coupled to the growth hormone drug, the oligomer further comprises one or more bonding moieties that are used to covalently couple the oligomer with the growth hormone drug as will be understood by those skilled in the art. Bonding moieties are preferably selected from the group consisting of covalent bond(s), ester moieties, carbonate moieties, carbamate moieties, amide moieties and secondary amine moieties. More than one moiety on the oligomer may be covalently coupled to the growth hormone drug.

While the oligomer is preferably covalently coupled to the growth hormone drug, it is to be understood that the oligomer may be non-covalently coupled to the growth hormone drug to form a non-covalently conjugated growth hormone drug-oligomer complex. As will be understood by those skilled in the art, non-covalent couplings include, but are not limited to, hydrogen bonding, ionic bonding, Van der Waals bonding, and micellular or liposomal encapsulation. According to embodiments of the present invention, oligomers may be suitably constructed, modified and/or appropriately functionalized to impart the ability for non-covalent conjugation in a selected manner (e.g., to impart hydrogen bonding capability), as will be understood by those skilled in the art. According to other embodiments of present invention, oligomers may be derivatized with various compounds including, but not limited

to, amino acids, oligopeptides, peptides, bile acids, bile acid derivatives, fatty acids, fatty acid derivatives, salicylic acids, salicylic acid derivatives, aminosalicylic acids, and aminosalicylic acid derivatives. The resulting oligomers can non-covalently couple (complex) with drug molecules, pharmaceutical products, and/or pharmaceutical excipients. The resulting complexes preferably have balanced lipophilic and hydrophilic properties. According to still other embodiments of the present invention, oligomers may be derivatized with amine and/or alkyl amines. Under suitable acidic conditions, the resulting oligomers can form non-covalently conjugated complexes with drug molecules, pharmaceutical products and/or pharmaceutical excipients. The products resulting from such complexation preferably have balanced lipophilic and hydrophilic properties.

More than one oligomer (i.e., a plurality of oligomers) may be coupled to the growth hormone drug. The oligomers in the plurality are preferably the same. However, it is to be understood that the oligomers in the plurality may be different from one another, or, alternatively, some of the oligomers in the plurality may be the same and some may be different. When a plurality of oligomers are coupled to the growth hormone drug, it may be preferable to couple one or more of the oligomers to the growth hormone drug with hydrolyzable bonds and couple one or more of the oligomers to the growth hormone drug with non-hydrolyzable bonds. Alternatively, all of the bonds coupling the plurality of oligomers to the growth hormone drug may be hydrolyzable, but have varying degrees of hydrolyzability such that, for example, one or more of the oligomers is rapidly removed from the growth hormone drug by hydrolysis in the body and one or more of the oligomers is slowly removed from the growth hormone drug by hydrolysis in the body.

The oligomer may be coupled to the growth hormone drug at various nucleophilic residues of the drug including, but not limited to, nucleophilic hydroxyl functions and/or amino functions. Nucleophilic hydroxyl functions may be found, for example, at serine and/or tyrosine residues, and nucleophilic amino functions may be found, for example, at histidine and/or lysine residues, and/or at the one or more N-termini of the polypeptide. When an oligomer is coupled to the one or more N-termini of the growth hormone polypeptide, the coupling preferably forms a secondary amine. When the growth hormone drug is human growth hormone, for example, the oligomer may be coupled to an amino functionality of Phe^{1}, Lys^{38}, Lys^{41}, Lys^{70}, Lys^{115}, Lys^{140}, Lys^{145}, Lys^{158}, Lys^{168}, and/or Lys^{172}.

Mixtures of growth hormone drug-oligomer conjugates having a dispersity coefficient greater than 10,000 may be synthesized by various methods. For example, a mixture of oligomers having a dispersity coefficient greater than 10,000 consisting of carboxylic acid and polyethylene glycol is synthesized by contacting a mixture of carboxylic acid having a dispersity coefficient greater than 10,000 with a mixture of polyethylene glycol having a dispersity coefficient greater than 10,000 under conditions sufficient to provide a mixture of oligomers having a dispersity coefficient greater than 10,000. The oligomers of the mixture having a dispersity coefficient greater than 10,000 are then activated so that they are capable of reacting with a growth hormone drug to provide a growth hormone drug-oligomer conjugate. One embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 3** and described in Examples 11-18 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 4** and described in Examples 19-24 hereinbelow. Still another embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 5** and described in Examples 25-29 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 6** and described in Examples 30-31 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 7** and described in Examples 32-37 hereinbelow. Still another embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 8** and described in Example 38 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 9** and described in Example 39 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers having a dispersity coefficient greater than 10,000 is illustrated in **Figure 10** and described in Example 40 hereinbelow.

The mixture of activated oligomers having a dispersity coefficient greater than 10,000 is reacted with a mixture of growth hormone drugs having a dispersity coefficient greater than 10,000 under conditions sufficient to provide a mixture of growth hormone drug-oligomer conjugates. Exemplary syntheses are described hereinbelow in Examples 40

through 42. As will be understood by those skilled in the art, the reaction conditions (e.g., selected molar ratios, solvent mixtures and/or pH) may be controlled such that the mixture of growth hormone drug-oligomer conjugates resulting from the reaction of the mixture of activated oligomers having a dispersity coefficient greater than 10,000 and the mixture of growth hormone drugs having a dispersity coefficient greater than 10,000 is a mixture having a dispersity coefficient greater than 10,000. For example, conjugation at the amino functionality of lysine may be suppressed by maintaining the pH of the reaction solution below the pK_a of lysine. Alternatively, the mixture of growth hormone drug-oligomer conjugates may be separated and isolated utilizing, for example, HPLC to provide a mixture of growth hormone drug-oligomer conjugates, for example mono-, di-, or tri-conjugates, having a dispersity coefficient greater than 10,000. The degree of conjugation (e.g., whether the isolated molecule is a mono-, di-, or tri-conjugate) of a particular isolated conjugate may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, mass spectroscopy. The particular conjugate structure (e.g., whether the oligomer is at Phe^{1}, Lys^{38}, Lys^{41}, Lys^{70}, Lys^{115}, Lys^{140}, Lys^{145}, Lys^{158}, Lys^{168} or Lys^{172} of a human growth hormone monoconjugate) may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, sequence analysis, peptide mapping, selective enzymatic cleavage, and/or endopeptidase cleavage.

As will be understood by those skilled in the art, one or more of the reaction sites on the growth hormone drug may be blocked by, for example, reacting the growth hormone drug with a suitable blocking reagent such as N-tert-butoxycarbonyl (t-BOC), or N-(9-fluorenylmethoxycarbonyl) (N-FMOC). This process may be preferred, for example, when the growth hormone drug is a polypeptide and it is desired to form an unsaturated conjugate (i.e., a conjugate wherein not all nucleophilic residues are conjugated) having an oligomer at the one or more N-termini of the polypeptide. Following such blocking, the mixture of blocked growth hormone drugs having a dispersity coefficient greater than 10,000 may be reacted with the mixture of activated oligomers having a dispersity coefficient greater than 10,000 to provide a mixture of growth hormone drug-oligomer conjugates having oligomer(s) coupled to one or more nucleophilic residues and having blocking moieties coupled to other nucleophilic residues. After the conjugation reaction, the growth hormone drug-oligomer conjugates may be de-blocked as will be understood by those skilled in the art. If necessary,

the mixture of growth hormone drug-oligomer conjugates may then be separated as described above to provide a mixture of growth hormone drug-oligomer conjugates having a dispersity coefficient greater than 10,000. Alternatively, the mixture of growth hormone drug-oligomer conjugates may be separated prior to de-blocking.

Mixtures of growth hormone drug-oligomer conjugates having a dispersity coefficient greater than 10,000 according to embodiments of the present invention preferably have improved properties when compared with those of conventional mixtures. For example, a mixture of growth hormone drug-oligomer conjugates having a dispersity coefficient greater than 10,000 preferably has an *in vivo* activity that is greater than the *in vivo* activity of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the mixture of growth hormone drug-oligomer conjugates having a dispersity coefficient greater than 10,000. As will be understood by those skilled in the art, the number average molecular weight of the mixture of growth hormone drug-oligomer conjugates having a dispersity coefficient greater than 10,000 and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography such as gel permeation chromatography as described, for example, in H.R. Allcock & F.W. Lampe, CONTEMPORARY POLYMER CHEMISTRY 394-402 (2d. ed., 1991).

As another example, a mixture of growth hormone drug-oligomer conjugates having a dispersity coefficient greater than 10,000 preferably has an *in vitro* activity that is greater than the *in vitro* activity of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the mixture of growth hormone drug-oligomer conjugates having a dispersity coefficient greater than 10,000. As will be understood by those skilled in the art, the number average molecular weight of the mixture of growth hormone drug-oligomer conjugates having a dispersity coefficient greater than 10,000 and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

The *in vitro* activity of a particular mixture may be measured by various methods, as will be understood by those skilled in the art. Preferably, the *in vitro* activity is measured using a Cytosensor® Microphysiometer commercially available from Molecular Devices Corporation of Sunnyvale, California. The microphysiometer monitors small changes in the

rates of extracellular acidification in response to a drug being added to cultured cells in a transwell. This response is proportional to the activity of the molecule under study.

As still another example, a mixture of growth hormone drug-oligomer conjugates having a dispersity coefficient greater than 10,000 preferably has an increased resistance to degradation by chymotrypsin when compared to the resistance to degradation by chymotrypsin of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the mixture of growth hormone drug-oligomer conjugates having a dispersity coefficient greater than 10,000. As will be understood by those skilled in the art, the number average molecular weight of the mixture of growth hormone drug-oligomer conjugates having a dispersity coefficient greater than 10,000 and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

As yet another example, a mixture of growth hormone drug-oligomer conjugates having a dispersity coefficient greater than 10,000 preferably has an inter-subject variability that is less than the inter-subject variability of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the mixture of growth hormone drug-oligomer conjugates having a dispersity coefficient greater than 10,000. As will be understood by those skilled in the art, the number average molecular weight of the mixture of growth hormone drug-oligomer conjugates having a dispersity coefficient greater than 10,000 and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography. The inter-subject variability may be measured by various methods as will be understood by those skilled in the art. The inter-subject variability is preferably calculated as follows. The area under a dose response curve (AUC) (i.e., the area between the dose-response curve and a baseline value) is determined for each subject. The average AUC for all subjects is determined by summing the AUCs of each subject and dividing the sum by the number of subjects. The absolute value of the difference between the subject's AUC and the average AUC is then determined for each subject. The absolute values of the differences obtained are then summed to give a value that represents the inter-subject variability. Lower values represent lower inter-subject variabilities and higher values represent higher inter-subject variabilities.

Mixtures of growth hormone drug-oligomer conjugates having a dispersity coefficient greater than 10,000 according to embodiments of the present invention preferably have two or more of the above-described improved properties. More preferably, mixtures of growth hormone drug-oligomer conjugates having a dispersity coefficient greater than 10,000 according to embodiments of the present invention have three or more of the above-described improved properties. Most preferably, mixtures of growth hormone drug-oligomer conjugates having a dispersity coefficient greater than 10,000 according to embodiments of the present invention have all four of the above-described improved properties.

According to other embodiments of the present invention, a mixture of conjugates in which each conjugate includes a growth hormone drug coupled to an oligomer and has the same number of polyalkylene glycol subunits.

The growth hormone drug is preferably human growth hormone. However, it is to be understood that the growth hormone drug may be selected from various growth hormone drugs known to those skilled in the art including, for example, growth hormone peptides, growth hormone peptide analogues, growth hormone peptide fragments, and growth hormone peptide fragment analogues. Growth hormone peptides include, but are not limited to, growth hormone, human (hGH); growth hormone, porcine; growth hormone, bovine; growth hormone, chicken; growth hormone, rat; growth hormone, mouse; growth hormone, ovine; growth hormone releasing factor, human; growth hormone pro-releasing factor, human; growth hormone releasing factor, mouse; growth hormone releasing factor, ovine; growth hormone releasing factor, rat; growth hormone releasing factor, bovine; growth hormone releasing factor, porcine; and growth hormone releasing factor, chicken. Growth hormone peptide analogs may be provided as described above by substituting one or more amino acids in a growth hormone peptide. Growth hormone peptide fragments include, but are not limited to, growth hormone 1-43, human; growth hormone 6-13; growth hormone releasing factor 1-37, human; growth hormone releasing factor 1-40, human; growth hormone releasing factor 1-40, amide, human; growth hormone releasing factor 30-44, amide, human; growth hormone releasing factor 1-29, amide, rat; hexarelin (growth hormone releasing hexapeptide); and growth hormone releasing factor 1-29, amide, human. Growth hormone peptide fragment analogues include, but are not limited to, [D-Ala^2]-growth hormone releasing factor 1-29, amide, human; [N-Ac-Tyr^1, D-Arg^2]-growth hormone releasing factor 1-29, amide;

[His[1], Nle[27]]-growth hormone releasing factor 1-32, amide; growth hormone releasing peptide-6 ([His[1], Lys[6]]-GHRP); and [D-Lys[3]]-GHRP-6.

The oligomer may be various oligomers comprising a polyalkylene glycol moiety as will be understood by those skilled in the art. Preferably, the polyalkylene glycol moiety has at least 2, 3, or 4 polyalkylene glycol subunits. More preferably, the polyalkylene glycol moiety has at least 5 or 6 polyalkylene glycol subunits. Most preferably, the polyalkylene glycol moiety of the oligomer has at least 7 polyalkylene glycol subunits. The polyalkylene glycol moiety of the oligomer is preferably a lower alkyl polyalkylene glycol moiety such as a polyethylene glycol moiety, a polypropylene glycol moiety, or a polybutylene glycol moiety. The polyalkylene glycol moiety is more preferably a polypropylene glycol moiety having a uniform structure. An exemplary polypropylene glycol moiety having a uniform structure is as follows:

$$-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-$$

This uniform polypropylene glycol structure may be described as having only one methyl substituted carbon atom adjacent each oxygen atom in the polypropylene glycol chain. Such uniform polypropylene glycol moieties may exhibit both lipophilic and hydrophilic characteristics and thus be useful in providing amphiphilic growth hormone drug-oligomer conjugates without the use of lipophilic polymer moieties. Furthermore, coupling the secondary alcohol moiety of the polypropylene glycol moiety with a growth hormone drug may provide the growth hormone drug (e.g., human growth hormone) with improved resistance to degradation caused by enzymes such as trypsin and chymotrypsin found, for example, in the gut.

Uniform polypropylene glycol according to embodiments of the present invention is preferably synthesized as illustrated in **Figures 11** through **13**, which will now be described. As illustrated in **Figure 11**, 1,2-propanediol **53** is reacted with a primary alcohol blocking reagent to provide a secondary alcohol extension monomer **54**. The primary alcohol blocking reagent may be various primary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, silylchloride compounds such as t-butyldiphenylsilylchloride and t-butyldimethylsilylchloride, and esterification reagents such as Ac_2O. Preferably, the primary alcohol blocking reagent is a primary alcohol blocking reagent that is substantially non-reactive with secondary alcohols, such as t-

butyldiphenylsilylchloride or t-butyldimethylsilylchloride. The secondary alcohol extension monomer (**54**) may be reacted with methanesulfonyl chloride ($MeSO_2Cl$) to provide a primary extension alcohol monomer mesylate **55**.

Alternatively, the secondary alcohol extension monomer **54** may be reacted with a secondary alcohol blocking reagent to provide compound **56**. The secondary alcohol blocking reagent may be various secondary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, benzyl chloride. The compound **56** may be reacted with a B_1 de-blocking reagent to remove the blocking moiety B_1 and provide a primary alcohol extension monomer **57**. The B_1 de-blocking reagent may be selected from various de-blocking reagents as will be understood by one skilled in the art. When the primary alcohol has been blocked by forming an ester, the B_1 de-blocking reagent is a de-esterification reagent, such as a base (e.g., potassium carbonate). When the primary alcohol has been blocked using a silylchloride, the B_1 de-blocking reagent is preferably tetrabutylammonium fluoride (TBAF). The primary alcohol extension monomer **57** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension monomer mesylate **58**.

The primary alcohol extension monomer **54** and the secondary alcohol extension monomer **57** may be capped as follows. The secondary alcohol extension monomer **54** may be reacted with a capping reagent to provide a compound **59**. The capping reagent may be various capping reagents as will be understood by those skilled in the art including, but not limited to, alkyl halides such as methyl chloride. The compound **59** may be reacted with a B_1 de-blocking agent as described above to provide a primary alcohol capping monomer **60**. The primary alcohol capping monomer **60** may be reacted with methane sulfonyl chloride to provide the secondary alcohol capping monomer mesylate **61**. The primary alcohol extension monomer **57** may be reacted with a capping reagent to provide a compound **62**. The capping reagent may be various capping reagents as described above. The compound **62** may be reacted with a B_2 de-blocking reagent to remove the blocking moiety B_2 and provide a secondary alcohol capping monomer **63**. The B_2 de-blocking reagent may be various de-blocking agents as will be understood by those skilled in the art including, but not limited to, H_2 in the presence of a palladium/activated carbon catalyst. The secondary alcohol capping monomer may be reacted with methanesulfonyl chloride to provide a primary alcohol capping monomer mesylate **64**. While the embodiments illustrated in **Figure 11** show the

synthesis of capping monomers, it is to be understood that similar reactions may be performed to provide capping polymers.

In general, chain extensions may be effected by reacting a primary alcohol extension mono- or poly-mer such as the primary alcohol extension monomer **57** with a primary alcohol extension mono- or poly-mer mesylate such as the primary alcohol extension monomer mesylate **55** to provide various uniform polypropylene chains or by reacting a secondary alcohol extension mono- or poly-mer such as the secondary alcohol extension monomer **54** with a secondary alcohol extension mono-or poly-mer mesylate such as the secondary alcohol extension monomer mesylate **58**.

For example, in **Figure 13**, the primary alcohol extension monomer mesylate **55** is reacted with the primary alcohol extension monomer **57** to provide a dimer compound **65**. Alternatively, the secondary alcohol extension monomer mesylate **58** may be reacted with the secondary alcohol extension monomer **54** to provide the dimer compound **65**. The B_1 blocking moiety on the dimer compound **65** may be removed using a B_1 de-blocking reagent as described above to provide a primary alcohol extension dimer **66**. The primary alcohol extension dimer **66** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension dimer mesylate **67**. Alternatively, the B_2 blocking moiety on the dimer compound **65** may be removed using the B_2 de-blocking reagent as described above to provide a secondary alcohol extension dimer **69**. The secondary alcohol extension dimer **69** may be reacted with methane sulfonyl chloride to provide a primary alcohol extension dimer mesylate **70**.

As will be understood by those skilled in the art, the chain extension process may be repeated to achieve various other chain lengths. For example, as illustrated in **Figure 13**, the primary alcohol extension dimer **66** may be reacted with the primary alcohol extension dimer mesylate **70** to provide a tetramer compound **72**. As further illustrated in **Figure 13**, a generic chain extension reaction scheme involves reacting the primary alcohol extension mono- or poly-mer **73** with the primary alcohol extension mono- or poly-mer mesylate **74** to provide the uniform polypropylene polymer **75**. The values of m and n may each range from 0 to 1000 or more. Preferably, m and n are each from 0 to 50. While the embodiments illustrated in **Figure 13** show primary alcohol extension mono- and/or poly-mers being reacted with primary alcohol extension mono- and/or poly-mer mesylates, it is to be

understood that similar reactions may be carried out using secondary alcohol extension mono- and/or poly-mers and secondary alcohol extension mono- and/or poly-mer mesylates.

An end of a primary alcohol extension mono- or poly-mer or an end of a primary alcohol extension mono- or poly-mer mesylate may be reacted with a primary alcohol capping mono- or poly-mer mesylate or a primary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the primary alcohol extension dimer mesylate **70** is reacted with the primary alcohol capping monomer **60** to provide the capped/blocked primary alcohol extension trimer **71**. As will be understood by those skilled in the art, the B_1 blocking moiety may be removed and the resulting capped primary alcohol extension trimer may be reacted with a primary alcohol extension mono- or poly-mer mesylate to extend the chain of the capped trimer **71**.

An end of a secondary alcohol extension mono-or poly-mer or an end of a secondary alcohol extension mono-or poly-mer mesylate may be reacted with a secondary alcohol capping mono-or poly-mer mesylate or a secondary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the secondary alcohol extension dimer mesylate **67** is reacted with the secondary alcohol capping monomer **63** to provide the capped/blocked primary alcohol extension trimer **68**. The B_2 blocking moiety may be removed as described above and the resulting capped secondary alcohol extension trimer may be reacted with a secondary alcohol extension mer mesylate to extend the chain of the capped trimer **68**. While the syntheses illustrated in **Figure 12** show the reaction of a dimer with a capping monomer to provide a trimer, it is to be understood that the capping process may be performed at any point in the synthesis of a uniform polypropylene glycol moiety, or, alternatively, uniform polypropylene glycol moieties may be provided that are not capped. While the embodiments illustrated in **Figure 12** show the capping of a polybutylene oligomer by synthesis with a capping monomer, it is to be understood that polybutylene oligomers of the present invention may be capped directly (i.e., without the addition of a capping monomer) using a capping reagent as described above in **Figure 11**.

Uniform polypropylene glycol moieties according to embodiments of the present invention may be coupled to a growth hormone drug, a lipophilic moiety such as a carboxylic acid, and/or various other moieties by various methods as will be understood by those skilled

in the art including, but not limited to, those described herein with respect to polyethylene glycol moieties.

The oligomer may comprise one or more other moieties as will be understood by those skilled in the art including, but not limited to, hydrophilic moieties, lipophilic moieties, spacer moieties, linker moieties, and terminating moieties. The various moieties in the oligomer are covalently coupled to one another by either hydrolyzable or non-hydrolyzable bonds.

The oligomer may further comprise one or more hydrophilic moieties including, but not limited to, sugars, polyalkylene glycols, and polyamine/PEG copolymers. Adjacent polyalkylene glycol moieties will be considered to be the same moiety if they are coupled by an ether bond and have the same alkyl structure. For example, the moiety

$$—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—$$

is a single polyethylene glycol moiety having six polyethylene glycol subunits. Adjacent polyalkylene glycol moieties will be considered to be different moieties if they are coupled by a bond other than an ether bond or if they have different alkyl structures. For example, the moiety

$$—O—C_2H_4—O—C_2H_4—O—C_2H_4—O—C_2H_4—\overset{\displaystyle O}{\overset{\|}{C}}—O—C_2H_4—O—C_2H_4—$$

is a polyethylene glycol moiety having four polyethylene glycol subunits and a hydrophilic moiety having two polyethylene glycol subunits. Preferably, oligomers according to embodiments of the present invention comprise a polyalkylene glycol moiety and do not further comprise a hydrophilic moiety.

The oligomer may further comprise one or more lipophilic moieties as will be understood by those skilled in the art. The lipophilic moiety is preferably a saturated or unsaturated, linear or branched alkyl moiety or a saturated or unsaturated, linear or branched fatty acid moiety. When the lipophilic moiety is an alkyl moiety, it is preferably a linear, saturated or unsaturated alkyl moiety having 1 to 28 carbon atoms. More preferably, the alkyl moiety has 2 to 12 carbon atoms. When the lipophilic moiety is a fatty acid moiety, it is preferably a natural fatty acid moiety that is linear, saturated or unsaturated, having 2 to 18 carbon atoms. More preferably, the fatty acid moiety has 3 to 14 carbon atoms. Most preferably, the fatty acid moiety has at least 4, 5 or 6 carbon atoms.

The oligomer may further comprise one or more spacer moieties as will be understood by those skilled in the art. Spacer moieties may, for example, be used to separate a hydrophilic moiety from a lipophilic moiety, to separate a lipophilic moiety or hydrophilic moiety from the growth hormone drug, to separate a first hydrophilic or lipophilic moiety from a second hydrophilic or lipophilic moiety, or to separate a hydrophilic moiety or lipophilic moiety from a linker moiety. Spacer moieties are preferably selected from the group consisting of sugar, cholesterol and glycerine moieties.

The oligomer may further comprise one or more linker moieties that are used to couple the oligomer with the growth hormone drug as will be understood by those skilled in the art. Linker moieties are preferably selected from the group consisting of alkyl and fatty acid moieties.

The oligomer may further comprise one or more terminating moieties at the one or more ends of the oligomer which are not coupled to the growth hormone drug. The terminating moiety is preferably an alkyl or alkoxy moiety, and is more preferably a lower alkyl or lower alkoxy moiety. Most preferably, the terminating moiety is methyl or methoxy. While the terminating moiety is preferably an alkyl or alkoxy moiety, it is to be understood that the terminating moiety may be various moieties as will be understood by those skilled in the art including, but not limited to, sugars, cholesterol, alcohols, and fatty acids.

The oligomer is preferably covalently coupled to the growth hormone drug. In some embodiments, the growth hormone drug is coupled to the oligomer utilizing a hydrolyzable bond (e.g., an ester or carbonate bond). A hydrolyzable coupling may provide a growth hormone drug-oligomer conjugate that acts as a prodrug. In certain instances, for example where the growth hormone drug-oligomer conjugate is inactive (i.e., the conjugate lacks the ability to affect the body through the growth hormone drug's primary mechanism of action), a hydrolyzable coupling may provide for a time-release or controlled-release effect, administering the growth hormone drug over a given time period as one or more oligomers are cleaved from their respective growth hormone drug-oligomer conjugates to provide the active drug. In other embodiments, the growth hormone drug is coupled to the oligomer utilizing a non-hydrolyzable bond (e.g., a carbamate, amide, or ether bond). Use of a non-hydrolyzable bond may be preferable when it is desirable to allow the growth hormone drug-oligomer conjugate to circulate in the bloodstream for an extended period of time, preferably at least 2 hours. When the oligomer is covalently coupled to the growth hormone drug, the

oligomer further comprises one or more bonding moieties that are used to covalently couple the oligomer with the growth hormone drug as will be understood by those skilled in the art. Bonding moieties are preferably selected from the group consisting of covalent bond(s), ester moieties, carbonate moieties, carbamate moieties, amide moieties and secondary amine moieties. More than one moiety on the oligomer may be covalently coupled to the growth hormone drug.

While the oligomer is preferably covalently coupled to the growth hormone drug, it is to be understood that the oligomer may be non-covalently coupled to the growth hormone drug to form a non-covalently conjugated growth hormone drug-oligomer complex. As will be understood by those skilled in the art, non-covalent couplings include, but are not limited to, hydrogen bonding, ionic bonding, Van der Waals bonding, and micellular or liposomal encapsulation. According to embodiments of the present invention, oligomers may be suitably constructed, modified and/or appropriately functionalized to impart the ability for non-covalent conjugation in a selected manner (e.g., to impart hydrogen bonding capability), as will be understood by those skilled in the art. According to other embodiments of present invention, oligomers may be derivatized with various compounds including, but not limited to, amino acids, oligopeptides, peptides, bile acids, bile acid derivatives, fatty acids, fatty acid derivatives, salicylic acids, salicylic acid derivatives, aminosalicylic acids, and aminosalicylic acid derivatives. The resulting oligomers can non-covalently couple (complex) with drug molecules, pharmaceutical products, and/or pharmaceutical excipients. The resulting complexes preferably have balanced lipophilic and hydrophilic properties. According to still other embodiments of the present invention, oligomers may be derivatized with amine and/or alkyl amines. Under suitable acidic conditions, the resulting oligomers can form non-covalently conjugated complexes with drug molecules, pharmaceutical products and/or pharmaceutical excipients. The products resulting from such complexation preferably have balanced lipophilic and hydrophilic properties.

More than one oligomer (i.e., a plurality of oligomers) may be coupled to the growth hormone drug. The oligomers in the plurality are preferably the same. However, it is to be understood that the oligomers in the plurality may be different from one another, or, alternatively, some of the oligomers in the plurality may be the same and some may be different. When a plurality of oligomers are coupled to the growth hormone drug, it may be preferable to couple one or more of the oligomers to the growth hormone drug with

hydrolyzable bonds and couple one or more of the oligomers to the growth hormone drug with non-hydrolyzable bonds. Alternatively, all of the bonds coupling the plurality of oligomers to the growth hormone drug may be hydrolyzable, but have varying degrees of hydrolyzability such that, for example, one or more of the oligomers is rapidly removed from the growth hormone drug by hydrolysis in the body and one or more of the oligomers is slowly removed from the growth hormone drug by hydrolysis in the body.

The oligomer may be coupled to the growth hormone drug at various nucleophilic residues of the drug including, but not limited to, nucleophilic hydroxyl functions and/or amino functions. Nucleophilic hydroxyl functions may be found, for example, at serine and/or tyrosine residues, and nucleophilic amino functions may be found, for example, at histidine and/or lysine residues, and/or at the one or more N-termini of the polypeptide. When an oligomer is coupled to the one or more N-termini of the growth hormone polypeptide, the coupling preferably forms a secondary amine. When the growth hormone drug is human growth hormone, for example, the oligomer may be coupled to an amino functionality of Phe^{1}, Lys^{38}, Lys^{41}, Lys^{70}, Lys^{115}, Lys^{140}, Lys^{145}, Lys^{158}, Lys^{168}, and/or Lys^{172}.

Mixtures of growth hormone drug oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits may be synthesized by various methods. For example, a mixture of oligomers consisting of carboxylic acid and polyethylene glycol where each oligomer in the mixture has the same number of polyethylene glycol subunits is synthesized by contacting a mixture of carboxylic acid with a mixture of polyethylene glycol where each polyethylene glycol molecule in the mixture has the same number of polyethylene glycol subunits under conditions sufficient to provide a mixture of oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits. The oligomers of the mixture where each oligomer in the mixture has the same number of polyethylene glycol subunits are then activated so that they are capable of reacting with a growth hormone drug to provide a growth hormone drug-oligomer conjugate. One embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 3** and described in Examples 11-18 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 4** and described in Examples 19-24 hereinbelow. Still another embodiment of a synthesis route for providing a

mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 5** and described in Examples 25-29 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 6** and described in Examples 30-31 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 7** and described in Examples 32-37 hereinbelow. Still another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 8** and described in Example 38 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 9** and described in Example 39 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers having a mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is illustrated in **Figure 10** and described in Example 40 hereinbelow.

The mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits is reacted with a mixture of growth hormone drugs under conditions sufficient to provide a mixture of growth hormone drug-oligomer conjugates. Exemplary syntheses are described hereinbelow in Examples 40 through 42. As will be understood by those skilled in the art, the reaction conditions (e.g., selected molar ratios, solvent mixtures and/or pH) may be controlled such that the mixture of growth hormone drug-oligomer conjugates resulting from the reaction of the mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits and the mixture of growth hormone drugs is a mixture of conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits. For example, conjugation at the amino functionality of lysine may be suppressed by maintaining the pH of the reaction solution below the pK_a of lysine. Alternatively, the mixture of growth hormone drug-oligomer conjugates may be separated and isolated utilizing, for example, HPLC to provide a mixture of growth hormone drug-oligomer conjugates, for example mono-, di-, or tri-conjugates, where each conjugate in the mixture has the same number of polyethylene

glycol subunits. The degree of conjugation (e.g., whether the isolated molecule is a mono-, di-, or tri-conjugate) of a particular isolated conjugate may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, mass spectroscopy. The particular conjugate structure (e.g., whether the oligomer is at Phe^{1}, Lys^{38}, Lys^{41}, Lys^{70}, Lys^{115}, Lys^{140}, Lys^{145}, Lys^{158}, Lys^{168} or Lys^{172} of a human growth hormone monoconjugate) may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, sequence analysis, peptide mapping, selective enzymatic cleavage, and/or endopeptidase cleavage.

As will be understood by those skilled in the art, one or more of the reaction sites on the growth hormone drug may be blocked by, for example, reacting the growth hormone drug with a suitable blocking reagent such as N-tert-butoxycarbonyl (t-BOC), or N-(9-fluorenylmethoxycarbonyl) (N-FMOC). This process may be preferred, for example, when the growth hormone drug is a polypeptide and it is desired to form an unsaturated conjugate (i.e., a conjugate wherein not all nucleophilic residues are conjugated) having an oligomer at the one or more N-termini of the polypeptide. Following such blocking, the mixture of blocked growth hormone drugs may be reacted with the mixture of activated oligomers where each oligomer in the mixture has the same number of polyethylene glycol subunits to provide a mixture of growth hormone drug-oligomer conjugates having oligomer(s) coupled to one or more nucleophilic residues and having blocking moieties coupled to other nucleophilic residues. After the conjugation reaction, the growth hormone drug-oligomer conjugates may be de-blocked as will be understood by those skilled in the art. If necessary, the mixture of growth hormone drug-oligomer conjugates may then be separated as described above to provide a mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits. Alternatively, the mixture of growth hormone drug-oligomer conjugates may be separated prior to de-blocking.

Mixtures of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits according to embodiments of the present invention preferably have improved properties when compared with those of conventional mixtures. For example, a mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits preferably has an *in vivo* activity that is greater than the *in vivo* activity of a polydispersed

mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits. As will be understood by those skilled in the art, the number average molecular weight of the mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography such as gel permeation chromatography as described, for example, in H.R. Allcock & F.W. Lampe, CONTEMPORARY POLYMER CHEMISTRY 394-402 (2d. ed., 1991).

As another example, a mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits preferably has an *in vitro* activity that is greater than the *in vitro* activity of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits. As will be understood by those skilled in the art, the number average molecular weight of the mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

The *in vitro* activity of a particular mixture may be measured by various methods, as will be understood by those skilled in the art. Preferably, the *in vitro* activity is measured using a Cytosensor® Microphysiometer commercially available from Molecular Devices Corporation of Sunnyvale, California. The microphysiometer monitors small changes in the rates of extracellular acidification in response to a drug being added to cultured cells in a transwell. This response is proportional to the activity of the molecule under study.

As still another example, a mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits according to still other embodiments of the present invention has an increased resistance to degradation by chymotrypsin when compared to the resistance to degradation by chymotrypsin of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the mixture of growth hormone drug-

oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits. As will be understood by those skilled in the art, the number average molecular weight of the mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

As yet another example, a mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits according to yet other embodiments of the present invention has an inter-subject variability that is less than the inter-subject variability of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits. As will be understood by those skilled in the art, the number average molecular weight of the mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography. The inter-subject variability may be measured by various methods as will be understood by those skilled in the art. The inter-subject variability is preferably calculated as follows. The area under a dose response curve (AUC) (i.e., the area between the dose-response curve and a baseline value) is determined for each subject. The average AUC for all subjects is determined by summing the AUCs of each subject and dividing the sum by the number of subjects. The absolute value of the difference between the subject's AUC and the average AUC is then determined for each subject. The absolute values of the differences obtained are then summed to give a value that represents the inter-subject variability. Lower values represent lower inter-subject variabilities and higher values represent higher inter-subject variabilities.

Mixtures of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits according to embodiments of the present invention preferably have two or more of the above-described improved properties. More preferably, mixtures of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits according

to embodiments of the present invention have three or more of the above-described improved properties. Most preferably, mixtures of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same number of polyethylene glycol subunits according to embodiments of the present invention have all four of the above-described improved properties.

According to still other embodiments of the present invention, a mixture of conjugates is provided in which each conjugate has the same molecular weight and has the formula:

$$\text{Growth Hormone Drug}\left[B-L_h-G_i-R_m-G'_j-R'_n-G''_k-T\right]_p \qquad (A)$$

wherein:

B is a bonding moiety;

L is a linker moiety;

G, G' and G" are individually selected spacer moieties;

R is a lipophilic moiety and R' is a polyalkylene glycol moiety, or R' is the lipophilic moiety and R is the polyalkylene glycol moiety;

T is a terminating moiety;

h, i, j, k, m and n are individually 0 or 1, with the proviso that when R is the polyalkylene glycol moiety; m is 1, and when R' is the polyalkylene glycol moiety, n is 1; and

p is an integer from 1 to the number of nucleophilic residues on the growth hormone drug.

The growth hormone drug is preferably human growth hormone. However, it is to be understood that the growth hormone drug may be selected from various growth hormone drugs known to those skilled in the art including, for example, growth hormone peptides, growth hormone peptide analogues, growth hormone peptide fragments, and growth hormone peptide fragment analogues. Growth hormone peptides include, but are not limited to, growth hormone, human (hGH); growth hormone, porcine; growth hormone, bovine; growth hormone, chicken; growth hormone, rat; growth hormone, mouse; growth hormone, ovine; growth hormone releasing factor, human; growth hormone pro-releasing factor, human; growth hormone releasing factor, mouse; growth hormone releasing factor, ovine; growth hormone releasing factor, rat; growth hormone releasing factor, bovine; growth hormone releasing factor, porcine; and growth hormone releasing factor, chicken. Growth hormone

peptide analogs may be provided as described above by substituting one or more amino acids in a growth hormone peptide. Growth hormone peptide fragments include, but are not limited to, growth hormone 1-43, human; growth hormone 6-13; growth hormone releasing factor 1-37, human; growth hormone releasing factor 1-40, human; growth hormone releasing factor 1-40, amide, human; growth hormone releasing factor 30-44, amide, human; growth hormone releasing factor 1-29, amide, rat; hexarelin (growth hormone releasing hexapeptide); and growth hormone releasing factor 1-29, amide, human. Growth hormone peptide fragment analogues include, but are not limited to, [D-Ala^{2}]-growth hormone releasing factor 1-29, amide, human; [N-Ac-Tyr^{1}, D-Arg^{2}]-growth hormone releasing factor 1-29, amide; [His^{1}, Nle^{27}]-growth hormone releasing factor 1-32, amide; growth hormone releasing peptide-6 ([His^{1}, Lys^{6}]-GHRP); and [D-Lys^{3}]-GHRP-6.

According to these embodiments of the present invention, R or R' is a polyalkylene moiety. Preferably, the polyalkylene glycol moiety has at least 2, 3, or 4 polyalkylene glycol subunits. More preferably, the polyalkylene glycol moiety has at least 5 or 6 polyalkylene glycol subunits. Most preferably, the polyalkylene glycol moiety of the oligomer has at least 7 polyalkylene glycol subunits. The polyalkylene glycol moiety of the oligomer is preferably a lower alkyl polyalkylene glycol moiety such as a polyethylene glycol moiety, a polypropylene glycol moiety, or a polybutylene glycol moiety. The polyalkylene glycol moiety is more preferably a polypropylene glycol moiety having a uniform structure. An exemplary polypropylene glycol moiety having a uniform structure is as follows:

$$-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-O-$$

This uniform polypropylene glycol structure may be described as having only one methyl substituted carbon atom adjacent each oxygen atom in the polypropylene glycol chain. Such uniform polypropylene glycol moieties may exhibit both lipophilic and hydrophilic characteristics and thus be useful in providing amphiphilic growth hormone drug-oligomer conjugates without the use of lipophilic polymer moieties (i.e., the sum of m + n is 1). Furthermore, coupling the secondary alcohol moiety of the polypropylene glycol moiety with a growth hormone drug may provide the growth hormone drug (e.g., human growth hormone) with improved resistance to degradation caused by enzymes such as trypsin and chymotrypsin found, for example, in the gut.

Uniform polypropylene glycol according to embodiments of the present invention is preferably synthesized as illustrated in **Figures 11** through **13**, which will now be described. As illustrated in **Figure 11**, 1,2-propanediol **53** is reacted with a primary alcohol blocking reagent to provide a secondary alcohol extension monomer **54**. The primary alcohol blocking reagent may be various primary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, silylchloride compounds such as t-butyldiphenylsilylchloride and t-butyldimethylsilylchloride, and esterification reagents such as Ac_2O. Preferably, the primary alcohol blocking reagent is a primary alcohol blocking reagent that is substantially non-reactive with secondary alcohols, such as t-butyldiphenylsilylchloride or t-butyldimethylsilylchloride. The secondary alcohol extension monomer (**54**) may be reacted with methanesulfonyl chloride ($MeSO_2Cl$) to provide a primary extension alcohol monomer mesylate **55**.

Alternatively, the secondary alcohol extension monomer **54** may be reacted with a secondary alcohol blocking reagent to provide compound **56**. The secondary alcohol blocking reagent may be various secondary alcohol blocking reagents as will be understood by those skilled in the art including, but not limited to, benzyl chloride. The compound **56** may be reacted with a B_1 de-blocking reagent to remove the blocking moiety B_1 and provide a primary alcohol extension monomer **57**. The B_1 de-blocking reagent may be selected from various de-blocking reagents as will be understood by one skilled in the art. When the primary alcohol has been blocked by forming an ester, the B_1 de-blocking reagent is a de-esterification reagent, such as a base (e.g., potassium carbonate). When the primary alcohol has been blocked using a silylchloride, the B_1 de-blocking reagent is preferably tetrabutylammonium fluoride (TBAF). The primary alcohol extension monomer **57** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension monomer mesylate **58**.

The primary alcohol extension monomer **54** and the secondary alcohol extension monomer **57** may be capped as follows. The secondary alcohol extension monomer **54** may be reacted with a capping reagent to provide a compound **59**. The capping reagent may be various capping reagents as will be understood by those skilled in the art including, but not limited to, alkyl halides such as methyl chloride. The compound **59** may be reacted with a B_1 de-blocking agent as described above to provide a primary alcohol capping monomer **60**. The primary alcohol capping monomer **60** may be reacted with methane sulfonyl chloride to

provide the secondary alcohol capping monomer mesylate **61**. The primary alcohol extension monomer **57** may be reacted with a capping reagent to provide a compound **62**. The capping reagent may be various capping reagents as described above. The compound **62** may be reacted with a B_2 de-blocking reagent to remove the blocking moiety B_2 and provide a secondary alcohol capping monomer **63**. The B_2 de-blocking reagent may be various de-blocking agents as will be understood by those skilled in the art including, but not limited to, H_2 in the presence of a palladium/activated carbon catalyst. The secondary alcohol capping monomer may be reacted with methanesulfonyl chloride to provide a primary alcohol capping monomer mesylate **64**. While the embodiments illustrated in **Figure 11** show the synthesis of capping monomers, it is to be understood that similar reactions may be performed to provide capping polymers.

In general, chain extensions may be effected by reacting a primary alcohol extension mono- or poly-mer such as the primary alcohol extension monomer **57** with a primary alcohol extension mono- or poly-mer mesylate such as the primary alcohol extension monomer mesylate **55** to provide various uniform polypropylene chains or by reacting a secondary alcohol extension mono- or poly-mer such as the secondary alcohol extension monomer **54** with a secondary alcohol extension mono-or poly-mer mesylate such as the secondary alcohol extension monomer mesylate **58**.

For example, in **Figure 13**, the primary alcohol extension monomer mesylate **55** is reacted with the primary alcohol extension monomer **57** to provide a dimer compound **65**. Alternatively, the secondary alcohol extension monomer mesylate **58** may be reacted with the secondary alcohol extension monomer **54** to provide the dimer compound **65**. The B_1 blocking moiety on the dimer compound **65** may be removed using a B_1 de-blocking reagent as described above to provide a primary alcohol extension dimer **66**. The primary alcohol extension dimer **66** may be reacted with methane sulfonyl chloride to provide a secondary alcohol extension dimer mesylate **67**. Alternatively, the B_2 blocking moiety on the dimer compound **65** may be removed using the B_2 de-blocking reagent as described above to provide a secondary alcohol extension dimer **69**. The secondary alcohol extension dimer **69** may be reacted with methane sulfonyl chloride to provide a primary alcohol extension dimer mesylate **70**.

As will be understood by those skilled in the art, the chain extension process may be repeated to achieve various other chain lengths. For example, as illustrated in **Figure 13**, the

primary alcohol extension dimer **66** may be reacted with the primary alcohol extension dimer mesylate **70** to provide a tetramer compound **72**. As further illustrated in **Figure 13**, a generic chain extension reaction scheme involves reacting the primary alcohol extension mono- or poly-mer **73** with the primary alcohol extension mono- or poly-mer mesylate **74** to provide the uniform polypropylene polymer **75**. The values of m and n may each range from 0 to 1000 or more. Preferably, m and n are each from 0 to 50. While the embodiments illustrated in **Figure 13** show primary alcohol extension mono- and/or poly-mers being reacted with primary alcohol extension mono- and/or poly-mer mesylates, it is to be understood that similar reactions may be carried out using secondary alcohol extension mono- and/or poly-mers and secondary alcohol extension mono- and/or poly-mer mesylates.

An end of a primary alcohol extension mono- or poly-mer or an end of a primary alcohol extension mono- or poly-mer mesylate may be reacted with a primary alcohol capping mono- or poly-mer mesylate or a primary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the primary alcohol extension dimer mesylate **70** is reacted with the primary alcohol capping monomer **60** to provide the capped/blocked primary alcohol extension trimer **71**. As will be understood by those skilled in the art, the B_1 blocking moiety may be removed and the resulting capped primary alcohol extension trimer may be reacted with a primary alcohol extension mono- or poly-mer mesylate to extend the chain of the capped trimer **71**.

An end of a secondary alcohol extension mono-or poly-mer or an end of a secondary alcohol extension mono-or poly-mer mesylate may be reacted with a secondary alcohol capping mono-or poly-mer mesylate or a secondary alcohol capping mono- or poly-mer, respectively, to provide a capped uniform polypropylene chain. For example, as illustrated in **Figure 12**, the secondary alcohol extension dimer mesylate **67** is reacted with the secondary alcohol capping monomer **63** to provide the capped/blocked primary alcohol extension trimer **68**. The B_2 blocking moiety may be removed as described above and the resulting capped secondary alcohol extension trimer may be reacted with a secondary alcohol extension mer mesylate to extend the chain of the capped trimer **68**. While the syntheses illustrated in **Figure 12** show the reaction of a dimer with a capping monomer to provide a trimer, it is to be understood that the capping process may be performed at any point in the synthesis of a uniform polypropylene glycol moiety, or, alternatively, uniform polypropylene glycol moieties may be provided that are not capped. While the embodiments illustrated in **Figure**

12 show the capping of a polybutylene oligomer by synthesis with a capping monomer, it is to be understood that polybutylene oligomers of the present invention may be capped directly (i.e., without the addition of a capping monomer) using a capping reagent as described above in **Figure 11**.

Uniform polypropylene glycol moieties according to embodiments of the present invention may be coupled to a growth hormone drug, a lipophilic moiety such as a carboxylic acid, and/or various other moieties by various methods as will be understood by those skilled in the art including, but not limited to, those described herein with respect to polyethylene glycol moieties.

According to these embodiments of the present invention, R or R' is a lipophilic moiety as will be understood by those skilled in the art. The lipophilic moiety is preferably a saturated or unsaturated, linear or branched alkyl moiety or a saturated or unsaturated, linear or branched fatty acid moiety. When the lipophilic moiety is an alkyl moiety, it is preferably a linear, saturated or unsaturated alkyl moiety having 1 to 28 carbon atoms. More preferably, the alkyl moiety has 2 to 12 carbon atoms. When the lipophilic moiety is a fatty acid moiety, it is preferably a natural fatty acid moiety that is linear, saturated or unsaturated, having 2 to 18 carbon atoms. More preferably, the fatty acid moiety has 3 to 14 carbon atoms. Most preferably, the fatty acid moiety has at least 4, 5 or 6 carbon atoms.

According to these embodiments of the present invention, the spacer moieties, G, G' and G", are spacer moieties as will be understood by those skilled in the art. Spacer moieties are preferably selected from the group consisting of sugar, cholesterol and glycerine moieties. Preferably, oligomers of these embodiments do not include spacer moieties (i.e., i, j and k are preferably 0).

According to these embodiments of the present invention, the linker moiety, L, may be used to couple the oligomer with the growth hormone drug as will be understood by those skilled in the art. Linker moieties are preferably selected from the group consisting of alkyl and fatty acid.

According to these embodiments of the present invention, the terminating moiety is preferably an alkyl or alkoxy moiety, and is more preferably a lower alkyl or lower alkoxy moiety. Most preferably, the terminating moiety is methyl or methoxy. While the terminating moiety is preferably an alkyl or alkoxy moiety, it is to be understood that the

terminating moiety may be various moieties as will be understood by those skilled in the art including, but not limited to, sugars, cholesterol, alcohols, and fatty acids.

According to these embodiments of the present invention, the oligomer, which is represented by the bracketed portion of the structure of Formula A, is covalently coupled to the growth hormone drug. In some embodiments, the growth hormone drug is coupled to the oligomer utilizing a hydrolyzable bond (e.g., an ester or carbonate bond). A hydrolyzable coupling may provide a growth hormone drug-oligomer conjugate that acts as a prodrug. In certain instances, for example where the growth hormone drug-oligomer conjugate is inactive (i.e., the conjugate lacks the ability to affect the body through the growth hormone drug's primary mechanism of action), a hydrolyzable coupling may provide for a time-release or controlled-release effect, administering the growth hormone drug over a given time period as one or more oligomers are cleaved from their respective growth hormone drug-oligomer conjugates to provide the active drug. In other embodiments, the growth hormone drug is coupled to the oligomer utilizing a non-hydrolyzable bond (e.g., a carbamate, amide, or ether bond). Use of a non-hydrolyzable bond may be preferable when it is desirable to allow the growth hormone drug-oligomer conjugate to circulate in the bloodstream for an extended period of time, preferably at least 2 hours. The bonding moiety may be various bonding moieties that may be used to covalently couple the oligomer with the growth hormone drug as will be understood by those skilled in the art. Bonding moieties are preferably selected from the group consisting of covalent bond(s), ester moieties, carbonate moieties, carbamate moieties, amide moieties and secondary amine moieties.

The variable p is an integer from 1 to the number of nucleophilic residues on the growth hormone drug. When p is greater than 1, more than one oligomer (i.e., a plurality of oligomers) is coupled to the drug. According to these embodiments of the present invention, the oligomers in the plurality are the same.

The oligomer may be coupled to the growth hormone drug at various nucleophilic residues of the drug including, but not limited to, nucleophilic hydroxyl functions and/or amino functions. Nucleophilic hydroxyl functions may be found, for example, at serine and/or tyrosine residues, and nucleophilic amino functions may be found, for example, at histidine and/or lysine residues, and/or at the one or more N-termini of the polypeptide. When an oligomer is coupled to the one or more N-termini of the growth hormone polypeptide, the coupling preferably forms a secondary amine. When the growth hormone

drug is human growth hormone, for example, the oligomer may be coupled to an amino functionality of Phe^{1}, Lys^{38}, Lys^{41}, Lys^{70}, Lys^{115}, Lys^{140}, Lys^{145}, Lys^{158}, Lys^{168}, and/or Lys^{172}.

Mixtures of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A may be synthesized by various methods. For example, a mixture of oligomers consisting of carboxylic acid and polyethylene glycol is synthesized by contacting a mixture of carboxylic acid with a mixture of polyethylene glycol under conditions sufficient to provide a mixture of oligomers. The oligomers of the mixture are then activated so that they are capable of reacting with a growth hormone drug to provide a growth hormone drug-oligomer conjugate. One embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 3** and described in Examples 11-18 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 4** and described in Examples 19-24 hereinbelow. Still another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 5** and described in Examples 25-29 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 6** and described in Examples 30-31 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 7** and described in Examples 32-37 hereinbelow. Still another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 8** and described in Example 38 hereinbelow. Yet another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is illustrated in **Figure 9** and described in Example 39 hereinbelow. Another embodiment of a synthesis route for providing a mixture of activated oligomers where each oligomer has the same molecular

weight and has a structure of the oligomer of Formula A is illustrated in **Figure 10** and described in Example 40 hereinbelow.

The mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A is reacted with a mixture of growth hormone drugs where each drug in the mixture has the same molecular weight under conditions sufficient to provide a mixture of growth hormone drug-oligomer conjugates. Exemplary syntheses are described hereinbelow in Examples 40 through 42. As will be understood by those skilled in the art, the reaction conditions (e.g., selected molar ratios, solvent mixtures and/or pH) may be controlled such that the mixture of growth hormone drug-oligomer conjugates resulting from the reaction of the mixture of activated oligomers where each oligomer has the same molecular weight and has a structure of the oligomer of Formula A and the mixture of growth hormone drugs is a mixture of conjugates where each conjugate has the same molecular weight and has the structure Formula A. For example, conjugation at the amino functionality of lysine may be suppressed by maintaining the pH of the reaction solution below the pK_a of lysine. Alternatively, the mixture of growth hormone drug-oligomer conjugates may be separated and isolated utilizing, for example, HPLC to provide a mixture of growth hormone drug-oligomer conjugates, for example mono-, di-, or tri-conjugates, where each conjugate in the mixture has the same number molecular weight and has the structure of Formula A. The degree of conjugation (e.g., whether the isolated molecule is a mono-, di-, or tri-conjugate) of a particular isolated conjugate may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, mass spectroscopy. The particular conjugate structure (e.g., whether the oligomer is at Phe^1, Lys^{38}, Lys^{41}, Lys^{70}, Lys^{115}, Lys^{140}, Lys^{145}, Lys^{158}, Lys^{168} or Lys^{172} of a human growth hormone monoconjugate) may be determined and/or verified utilizing various techniques as will be understood by those skilled in the art including, but not limited to, sequence analysis, peptide mapping, selective enzymatic cleavage, and/or endopeptidase cleavage.

As will be understood by those skilled in the art, one or more of the reaction sites on the growth hormone drug may be blocked by, for example, reacting the growth hormone drug with a suitable blocking reagent such as N-tert-butoxycarbonyl (t-BOC), or N-(9-fluorenylmethoxycarbonyl) (N-FMOC). This process may be preferred, for example, when the growth hormone drug is a polypeptide and it is desired to form an unsaturated conjugate

(i.e., a conjugate wherein not all nucleophilic residues are conjugated) having an oligomer at the one or more N-termini of the polypeptide. Following such blocking, the mixture of blocked growth hormone drugs may be reacted with the mixture of activated oligomers where each oligomer in the mixture has the same molecular weight and has a structure of the oligomer of Formula A to provide a mixture of growth hormone drug-oligomer conjugates having oligomer(s) coupled to one or more nucleophilic residues and having blocking moieties coupled to other nucleophilic residues. After the conjugation reaction, the growth hormone drug-oligomer conjugates may be de-blocked as will be understood by those skilled in the art. If necessary, the mixture of growth hormone drug-oligomer conjugates may then be separated as described above to provide a mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A. Alternatively, the mixture of growth hormone drug-oligomer conjugates may be separated prior to de-blocking.

Mixtures of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A according to embodiments of the present invention preferably have improved properties when compared with those of conventional mixtures. For example, a mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A preferably has an *in vivo* activity that is greater than the *in vivo* activity of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A. As will be understood by those skilled in the art, the number average molecular weight of the mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography such as gel permeation chromatography as described, for example, in H.R. Allcock & F.W. Lampe, CONTEMPORARY POLYMER CHEMISTRY 394-402 (2d. ed., 1991).

As another example, a mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A preferably has an *in vitro* activity that is greater than the *in vitro* activity of a

polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A. As will be understood by those skilled in the art, the number average molecular weight of the mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

The *in vitro* activity of a particular mixture may be measured by various methods, as will be understood by those skilled in the art. Preferably, the *in vitro* activity is measured using a Cytosensor® Microphysiometer commercially available from Molecular Devices Corporation of Sunnyvale, California. The microphysiometer monitors small changes in the rates of extracellular acidification in response to a drug being added to cultured cells in a transwell. This response is proportional to the activity of the molecule under study.

As still another example, a mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A preferably has an increased resistance to degradation by chymotrypsin when compared to the resistance to degradation by chymotrypsin of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A. As will be understood by those skilled in the art, the number average molecular weight of the mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography.

As yet another example, a mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A preferably has an inter-subject variability that is less than the inter-subject variability of a polydispersed mixture of growth hormone drug-oligomer conjugates having the same number average molecular weight as the mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the

structure of Formula A. As will be understood by those skilled in the art, the number average molecular weight of the mixture of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A and the number average weight of the polydispersed mixture may be measured by various methods including, but not limited to, size exclusion chromatography. The inter-subject variability may be measured by various methods as will be understood by those skilled in the art. The inter-subject variability is preferably calculated as follows. The area under a dose response curve (AUC) (i.e., the area between the dose-response curve and a baseline value) is determined for each subject. The average AUC for all subjects is determined by summing the AUCs of each subject and dividing the sum by the number of subjects. The absolute value of the difference between the subject's AUC and the average AUC is then determined for each subject. The absolute values of the differences obtained are then summed to give a value that represents the inter-subject variability. Lower values represent lower inter-subject variabilities and higher values represent higher inter-subject variabilities.

Mixtures of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A according to embodiments of the present invention preferably have two or more of the above-described improved properties. More preferably, mixtures of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A according to embodiments of the present invention have three or more of the above-described improved properties. Most preferably, mixtures of growth hormone drug-oligomer conjugates where each conjugate in the mixture has the same molecular weight and has the structure of Formula A according to embodiments of the present invention have all four of the above-described improved properties.

Pharmaceutical compositions comprising a conjugate mixture according to embodiments of the present invention are also provided. The mixtures of growth hormone drug-oligomer conjugates described above may be formulated for administration in a pharmaceutical carrier in accordance with known techniques. *See, e.g.,* Remington, *The Science And Practice of Pharmacy* (9^{th} Ed. 1995). In the manufacture of a pharmaceutical composition according to embodiments of the present invention, the mixture of growth hormone drug-oligomer conjugates is typically admixed with, *inter alia*, a pharmaceutically acceptable carrier. The carrier must, of course, be acceptable in the sense of being

compatible with any other ingredients in the pharmaceutical composition and should not be deleterious to the patient. The carrier may be a solid or a liquid, or both, and is preferably formulated with the mixture of growth hormone drug-oligomer conjugates as a unit-dose formulation, for example, a tablet, which may contain from about 0.01 or 0.5% to about 95% or 99% by weight of the mixture of growth hormone drug-oligomer conjugates. The pharmaceutical compositions may be prepared by any of the well known techniques of pharmacy including, but not limited to, admixing the components, optionally including one or more accessory ingredients.

The pharmaceutical compositions according to embodiments of the present invention include those suitable for oral, rectal, topical, inhalation (e.g., via an aerosol) buccal (e.g., sub-lingual), vaginal, parenteral (e.g., subcutaneous, intramuscular, intradermal, intraarticular, intrapleural, intraperitoneal, inracerebral, intraarterial, or intravenous), topical (i.e., both skin and mucosal surfaces, including airway surfaces) and transdermal administration, although the most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular mixture of growth hormone drug-oligomer conjugates which is being used.

Pharmaceutical compositions suitable for oral administration may be presented in discrete units, such as capsules, cachets, lozenges, or tables, each containing a predetermined amount of the mixture of growth hormone drug-oligomer conjugates; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Such formulations may be prepared by any suitable method of pharmacy which includes the step of bringing into association the mixture of growth hormone drug-oligomer conjugates and a suitable carrier (which may contain one or more accessory ingredients as noted above). In general, the pharmaceutical composition according to embodiments of the present invention are prepared by uniformly and intimately admixing the mixture of growth hormone drug-oligomer conjugates with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture. For example, a tablet may be prepared by compressing or molding a powder or granules containing the mixture of growth hormone drug-oligomer conjugates, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the mixture in a free-flowing form, such as a powder or granules optionally mixed with a binder, lubricant, inert diluent, and/or surface active/dispersing agent(s). Molded tablets may be

made by molding, in a suitable machine, the powdered compound moistened with an inert liquid binder.

Pharmaceutical compositions suitable for buccal (sub-lingual) administration include lozenges comprising the mixture of growth hormone drug-oligomer conjugates in a flavoured base, usually sucrose and acacia or tragacanth; and pastilles comprising the mixture of growth hormone drug-oligomer conjugates in an inert base such as gelatin and glycerin or sucrose and acacia.

Pharmaceutical compositions according to embodiments of the present invention suitable for parenteral administration comprise sterile aqueous and non-aqueous injection solutions of the mixture of growth hormone drug-oligomer conjugates, which preparations are preferably isotonic with the blood of the intended recipient. These preparations may contain anti-oxidants, buffers, bacteriostats and solutes which render the composition isotonic with the blood of the intended recipient. Aqueous and non-aqueous sterile suspensions may include suspending agents and thickening agents. The compositions may be presented in unit\dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline or water-for-injection immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described. For example, an injectable, stable, sterile composition comprising a mixture of growth hormone drug-oligomer conjugates in a unit dosage form in a sealed container may be provided. The mixture of growth hormone drug-oligomer conjugates is provided in the form of a lyophilizate which is capable of being reconstituted with a suitable pharmaceutically acceptable carrier to form a liquid composition suitable for injection thereof into a subject. The unit dosage form typically comprises from about 10 mg to about 10 grams of the mixture of growth hormone drug-oligomer conjugates. When the mixture of growth hormone drug-oligomer conjugates is substantially water-insoluble, a sufficient amount of emulsifying agent which is physiologically acceptable may be employed in sufficient quantity to emulsify the mixture of growth hormone drug-oligomer conjugates in an aqueous carrier. One such useful emulsifying agent is phosphatidyl choline.

Pharmaceutical compositions suitable for rectal administration are preferably presented as unit dose suppositories. These may be prepared by admixing the mixture of

growth hormone drug-oligomer conjugates with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

Pharmaceutical compositions suitable for topical application to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which may be used include petroleum jelly, lanoline, polyethylene glycols, alcohols, transdermal enhancers, and combinations of two or more thereof.

Pharmaceutical compositions suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Compositions suitable for transdermal administration may also be delivered by iontophoresis *(see,* for example, *Pharmaceutical Research* 3 **(6)**:318 (1986)) and typically take the form of an optionally buffered aqueous solution of the mixture of growth hormone drug-oligomer conjugates. Suitable formulations comprise citrate or bis\tris buffer (pH 6) or ethanol/water and contain from 0.1 to 0.2M active ingredient.

Methods of treating a growth hormone deficiency in a subject in need of such treatment by administering an effective amount of such pharmaceutical compositions are also provided. The effective amount of any mixture of growth hormone drug-oligomer conjugates, the use of which is in the scope of present invention, will vary somewhat from mixture to mixture, and patient to patient, and will depend upon factors such as the age and condition of the patient and the route of delivery. Such dosages can be determined in accordance with routine pharmacological procedures known to those skilled in the art. As a general proposition, a dosage from about 0.1 to about 50 mg/kg will have therapeutic efficacy, with all weights being calculated based upon the weight of the mixture of growth hormone drug-oligomer conjugates. Toxicity concerns at the higher level may restrict intravenous dosages to a lower level such as up to about 10 mg/kg, with all weights being calculated based upon the weight of the active base. A dosage from about 10 mg/kg to about 50 mg/kg may be employed for oral administration. Typically, a dosage from about 0.5 mg/kg to 5 mg/kg may be employed for intramuscular injection. The frequency of administration is usually one, two, or three times per day or as necessary to control the condition. Alternatively, the drug-oligomer conjugates may be administered by continuous infusion. The duration of treatment depends on the type of growth hormone deficiency being treated.

Methods of accelerating the growth rate of an animal comprise administering to the animal an effective amount of mixture of conjugates according to the various embodiments described above. The effective amount of growth hormone will, of course, depend upon the animal undergoing administration and can be determined by one of skill in the art.

Methods of synthesizing conjugate mixtures according to embodiments of the present invention are also provided. While the following embodiments of a synthesis route are directed to synthesis of a substantially monodispersed mixture, similar synthesis routes may be utilized for synthesizing other growth hormone drug-oligomer conjugate mixtures according to embodiments of the present invention.

A substantially monodispersed mixture of polymers comprising polyethylene glycol moieties is provided as illustrated in reaction **1**:

$$\underset{\textbf{(I)}}{R^1(OC_2H_4)_nO^-X^+} \quad + \quad \underset{\textbf{(II)}}{R^2(OC_2H_4)_mOMs} \longrightarrow \underset{\textbf{(III)}}{R^2(OC_2H_4)_{m+n}OR^1} \qquad \textbf{1}$$

R^1 is H or a lipophilic moiety. R^1 is preferably H, alkyl, aryl alkyl, an aromatic moiety, a fatty acid moiety, an ester of a fatty acid moiety, cholesteryl, or adamantyl. R^1 is more preferably H, lower alkyl, or an aromatic moiety. R^1 is most preferably H, methyl, or benzyl.

In Formula I, n is from 1 to 25. Preferably n is from 1 to 6.

X^+ is a positive ion. Preferably X^+ is any positive ion in a compound, such as a strong base, that is capable of ionizing a hydroxyl moiety on PEG. Examples of positive ions include, but are not limited to, sodium ions, potassium ions, lithium ions, cesium ions, and thallium ions.

R^2 is H or a lipophilic moiety. R^2 is preferably linear or branched alkyl, aryl alkyl, an aromatic moiety, a fatty acid moiety, or an ester of a fatty acid moiety. R^2 is more preferably lower alkyl, benzyl, a fatty acid moiety having 1 to 24 carbon atoms, or an ester of a fatty acid moiety having 1 to 24 carbon atoms. R^2 is most preferably methyl, a fatty acid moiety having 1 to 18 carbon atoms or an ethyl ester of a fatty acid moiety having 1 to 18 carbon atoms.

In Formula II, m is from 1 to 25. Preferably m is from 1 to 6.

Ms is a mesylate moiety (i.e., $CH_3S(O_2)$-).

As illustrated in reaction **1**, a mixture of compounds having the structure of Formula I is reacted with a mixture of compounds having the structure of Formula II to provide a

mixture of polymers comprising polyethylene glycol moieties and having the structure of Formula III. The mixture of compounds having the structure of Formula I is a substantially monodispersed mixture. Preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula I have the same molecular weight, and, more preferably, the mixture of compounds of Formula I is a monodispersed mixture. The mixture of compounds of Formula II is a substantially monodispersed mixture. Preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula II have the same molecular weight, and, more preferably, the mixture of compounds of Formula II is a monodispersed mixture. The mixture of compounds of Formula III is a substantially monodispersed mixture. Preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compound of Formula III have the same molecular weight. More preferably, the mixture of compounds of Formula III is a monodispersed mixture.

Reaction **1** is preferably performed between about 0°C and about 40°C, is more preferably performed between about 15°C and about 35°C, and is most preferably performed at room temperature (approximately 25°C).

Reaction **1** may be performed for various periods of time as will be understood by those skilled in the art. Reaction **1** is preferably performed for a period of time between about 0.25, 0.5 or 0.75 hours and about 2, 4 or 8 hours.

Reaction **1** is preferably carried out in an aprotic solvent such as, but not limited to, N,N-dimethylacetamide (DMA), N,N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), hexamethylphosphoric triamide, tetrahydrofuran (THF), dioxane, diethyl ether, methyl t-butyl ether (MTBE), toluene, benzene, hexane, pentane, N-methylpyrollidinone, tetrahydronaphthalene, decahydronaphthalene, 1,2-dichlorobenzene, 1,3-dimethyl-2-imidazolidinone, or a mixture thereof. More preferably, the solvent is DMF, DMA or toluene.

The molar ratio of the compound of Formula I to the compound of Formula II is preferably greater than about 1:1. More preferably, the molar ratio is at least about 2:1. By providing an excess of the compounds of Formula I, one can ensure that substantially all of the compounds of Formula II are reacted, which may aid in the recovery of the compounds of Formula III as discussed below.

Compounds of Formula I are preferably prepared as illustrated in reaction **2**:

$$R^1(OC_2H_4)_nOH \quad + \quad \underset{\text{Formula IV}}{\underset{\text{on the PEG moiety of}}{\underset{\text{ionizing a hydroxyl moiety}}{\text{compound capable of}}}} \longrightarrow \quad R^1(OC_2H_4)_nO^-X^+ \qquad \mathbf{2}$$

(IV) **(I)**

R^1 and X^+ are as described above and the mixture of compounds of Formula IV is substantially monodispersed; preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula IV have the same molecular weight; and, more preferably, the mixture of compounds of Formula IV is a monodispersed mixture.

Various compounds capable of ionizing a hydroxyl moiety on the PEG moiety of the compound of Formula IV will be understood by those skilled in the art. The compound capable of ionizing a hydroxyl moiety is preferably a strong base. More preferably, the compound capable of ionizing a hydroxyl moiety is selected from the group consisting of sodium hydride, potassium hydride, sodium t-butoxide, potassium t-butoxide, butyl lithium (BuLi), and lithium diisopropylamine. The compound capable of ionizing a hydroxyl moiety is more preferably sodium hydride.

The molar ratio of the compound capable of ionizing a hydroxyl moiety on the PEG moiety of the compound of Formula IV to the compound of Formula IV is preferably at least about 1:1, and is more preferably at least about 2:1. By providing an excess of the compound capable of ionizing the hydroxyl moiety, it is assured that substantially all of the compounds of Formula IV are reacted to provide the compounds of Formula I. Thus, separation difficulties, which may occur if both compounds of Formula IV and compounds of Formula I were present in the reaction product mixture, may be avoided.

Reaction **2** is preferably performed between about 0°C and about 40°C, is more preferably performed between about 0°C and about 35°C, and is most preferably performed between about 0°C and room temperature (approximately 25°C).

Reaction **2** may be performed for various periods of time as will be understood by those skilled in the art. Reaction **2** is preferably performed for a period of time between about 0.25, 0.5 or 0.75 hours and about 2, 4 or 8 hours.

Reaction **2** is preferably carried out in an aprotic solvent such as, but not limited to, N,N-dimethylacetamide (DMA), N,N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), hexamethylphosphoric triamide, tetrahydrofuran (THF), dioxane, diethyl ether, methyl t-butyl ether (MTBE), toluene, benzene, hexane, pentane, N-methylpyrollidinone, dichloromethane, chloroform, tetrahydronaphthalene, decahydronaphthalene, 1,2-

dichlorobenzene, 1,3-dimethyl-2-imidazolidinone, or a mixture thereof. More preferably, the solvent is DMF, dichloromethane or toluene.

Compounds of Formula II are preferably prepared as illustrated in reaction **3**:

$$\underset{\textbf{(V)}}{R^2(OC_2H_4)_mOH} \quad + \quad CH_3\overset{\overset{\large O}{\|}}{\underset{\underset{\large O}{\|}}{S}}Q \quad \longrightarrow \quad \underset{\textbf{(II)}}{R^2(OC_2H_4)_mOMs} \qquad \textbf{3}$$

R^2 and Ms are as described above and the compound of Formula V is present as a substantially monodispersed mixture of compounds of Formula V; preferably at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula V have the same molecular weight; and, more preferably, the mixture of compounds of Formula V is a monodispersed mixture.

Q is a halide, preferably chloride or fluoride.

$CH_3S(O_2)Q$ is methanesulfonyl halide. The methanesulfonyl halide is preferably methanesulfonyl chloride or methanesulfonyl fluoride. More preferably, the methanesulfonyl halide is methanesulfonyl chloride.

The molar ratio of the methane sulfonyl halide to the compound of Formula V is preferably greater than about 1:1, and is more preferably at least about 2:1. By providing an excess of the methane sulfonyl halide, it is assured that substantially all of the compounds of Formula V are reacted to provide the compounds of Formula II. Thus, separation difficulties, which may occur if both compounds of Formula V and compounds of Formula II were present in the reaction product mixture, may be avoided.

Reaction **3** is preferably performed between about -10°C and about 40°C, is more preferably performed between about 0°C and about 35°C, and is most preferably performed between about 0°C and room temperature (approximately 25°C).

Reaction **3** may be performed for various periods of time as will be understood by those skilled in the art. Reaction **3** is preferably performed for a period of time between about 0.25, 0.5 or 0.75 hours and about 2, 4 or 8 hours.

Reaction **3** is preferably carried out in the presence of an aliphatic amine including, but not limited to, monomethylamine, dimethylamine, trimethylamine, monoethylamine, diethylamine, triethylamine, monoisopropylamine, diisopropylamine, mono-n-butylamine, di-n-butylamine, tri-n-butylamine, monocyclohexylamine, dicyclohexylamine, or mixtures thereof. More preferably, the aliphatic amine is a tertiary amine such as triethylamine.

As will be understood by those skilled in the art, various substantially monodispersed mixtures of compounds of Formula V are commercially available. For example, when R^2 is H or methyl, the compounds of Formula V are PEG or mPEG compounds, respectively, which are commercially available from Aldrich of Milwaukee, Wisconsin; Fluka of Switzerland, and/or TCI America of Portland, Oregon.

When R^2 is a lipophilic moiety such as, for example, higher alkyl, fatty acid, an ester of a fatty acid, cholesteryl, or adamantyl, the compounds of Formula V may be provided by various methods as will be understood by those skilled in the art. The compounds of Formula V are preferably provided as follows:

$$\underset{\textbf{(VI)}}{R^2\text{—}OMs} \quad + \quad \underset{\textbf{(VII)}}{R^3(OC_2H_4)_m\text{–}O^-X_2^+} \longrightarrow \underset{\textbf{(VIII)}}{R^3(OC_2H_4)_m\text{–}OR^2} \qquad \mathbf{4}$$

$$\underset{\textbf{(VIII)}}{R^3(OC_2H_4)_m\text{–}OR^2} \longrightarrow \underset{\textbf{(V)}}{H(OC_2H_4)_m\text{–}OR^2} \qquad \mathbf{5}$$

R^2 is a lipophilic moiety, preferably higher alkyl, fatty acid ester, cholesteryl, or adamantyl, more preferably a lower alkyl ester of a fatty acid, and most preferably an ethyl ester of a fatty acid having from 1 to 18 carbon atoms.

R^3 is H, benzyl, trityl, tetrahydropyran, or other alcohol protecting groups as will be understood by those skilled in the art.

X_2^+ is a positive ion as described above with respect to X^+.

The value of m is as described above.

Regarding reaction **4**, a mixture of compounds of Formula VI is reacted with a mixture of compounds of Formula VII under reaction conditions similar to those described above with reference to reaction **1**. The mixture of compounds of Formula VI is a substantially monodispersed mixture. Preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula VI have the same molecular weight. More preferably, the mixture of compounds of Formula VI is a monodispersed mixture. The mixture of compounds of Formula VII is a substantially monodispersed mixture. Preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of

Formula VII have the same molecular weight. More preferably, the mixture of compounds of Formula VII is a monodispersed mixture.

Regarding reaction **5**, the compound of Formula VIII may be hydrolyzed to convert the R^3 moiety into an alcohol by various methods as will be understood by those skilled in the art. When R^3 is benzyl or trityl, the hydrolysis is preferably performed utilizing H_2 in the presence of a palladium-charcoal catalyst as is known by those skilled in the art. Of course, when R^3 is H, reaction **5** is unnecessary.

The compound of Formula VI may be commercially available or be provided as described above with reference to reaction **3**. The compound of Formula VII may be provided as described above with reference to reaction **2**.

Substantially monodispersed mixtures of polymers comprising PEG moieties and having the structure of Formula III above can further be reacted with other substantially monodispersed polymers comprising PEG moieties in order to extend the PEG chain. For example, the following scheme may be employed:

$$\underset{\textbf{(III)}}{R^2(OC_2H_4)_{m+n}-OR^1} \quad + \quad CH_3\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}Q \longrightarrow \underset{\textbf{(IX)}}{R^2(OC_2H_4)_{m+n}-OMs} \qquad \mathbf{6}$$

$$\underset{\textbf{(IX)}}{R^2(OC_2H_4)_{m+n}-OMs} \quad + \quad \underset{\textbf{(X)}}{R^4(OC_2H_4)_p-O^-X_2^+} \longrightarrow \underset{\textbf{(XI)}}{R^2(OC_2H_4)_{m+n+p}-OR^4} \qquad \mathbf{7}$$

Ms, m and n are as described above with reference to reaction **1**; p is similar to n and m, and X_2^+ is similar to X^+ as described above with reference to reaction **1**. Q is as described above with reference to reaction **3**. R^2 is as described above with reference to reaction **1** and is preferably lower alkyl. R^1 is H. Reaction **6** is preferably performed in a manner similar to that described above with reference to reaction **3**. Reaction **7** is preferably performed in a manner similar to that described above with reference to reaction **1**. Preferably, at least about 96, 97, 98 or 99 percent of the compounds in the mixture of compounds of Formula III have the same molecular weight, and, more preferably, the mixture of compounds of Formula III is a monodispersed mixture. The mixture of compounds of Formula X is a substantially monodispersed mixture. Preferably, at least about 96, 97, 98 or 99 percent of the compounds

in the mixture of compounds of Formula X have the same molecular weight, and, more preferably, the mixture of compounds of Formula X is a monodispersed mixture.

A process according to embodiments of the present invention is illustrated by the scheme shown in **Figure 1**, which will now be described. The synthesis of substantially monodispersed polyethylene glycol-containing oligomers begins by the preparation of the monobenzyl ether (**1**) of a substantially monodispersed polyethylene glycol. An excess of a commercially available substantially monodispersed polyethylene glycol is reacted with benzyl chloride in the presence of aqueous sodium hydroxide as described by Coudert *et al* (*Synthetic Communications*, **16(1)**: 19-26 (1986)). The sodium salt of **1** is then prepared by the addition of NaH, and this sodium salt is allowed to react with the mesylate synthesized from the ester of a hydroxyalkanoic acid (**2**). The product (**3**) of the displacement of the mesylate is debenzylated *via* catalytic hydrogenation to obtain the alcohol (**4**). The mesylate (**5**) of this alcohol may be prepared by addition of methanesulfonyl chloride and used as the electrophile in the reaction with the sodium salt of the monomethyl ether of a substantially monodispersed polyethylene glycol derivative, thereby extending the polyethylene glycol portion of the oligomer to the desired length, obtaining the elongated ester (**6**). The ester may be hydrolyzed to the acid (**7**) in aqueous base and transformed into the activated ester (**8**) by reaction with a carbodiimide and N-hydroxysuccinimide. While the oligomer illustrated in **Figure 1** is activated using N-hydroxysuccinimide, it is to be understood that various other reagents may be used to activate oligomers of the present invention including, but not limited to, active phenyl chloroformates such as *para*-nitrophenyl chloroformate, phenyl chloroformate, 3,4-phenyldichloroformate, and 3,4-phenyldichloroformate; tresylation; and acetal formation.

Still referring to **Figure 1**, q is from 1 to 24. Preferably, q is from 1 to 18, and q is more preferably from 4 to 16. R^4 is a moiety capable of undergoing hydrolysis to provide the carboxylic acid. R^4 is preferably lower alkyl and is more preferably ethyl. The variables n and m are as described above with reference to reaction **1**.

All starting materials used in the procedures described herein are either commercially available or can be prepared by methods known in the art using commercially available starting materials.

The present invention will now be described with reference to the following examples. It should be appreciated that these examples are for the purposes of illustrating

aspects of the present invention, and do not limit the scope of the invention as defined by the claims.

EXAMPLES

Examples 1 through 10

Reactions in Examples 1 through 10 were carried out under nitrogen with magnetic stirring, unless otherwise specified. "Work-up" denotes extraction with an organic solvent, washing of the organic phase with saturated NaCl solution, drying ($MgSO_4$), and evaporation (rotary evaporator). Thin layer chromatography was conducted with Merck glass plates precoated with silica gel 60°F - 254 and spots were visualized by iodine vapor. All mass spectra were determined by Macromolecular Resources Colorado State University, CO and are reported in the order m/z, (relative intensity). Elemental analyses and melting points were performed by Galbraith Laboratories, Inc., Knoxville, TN. Examples 1-10 refer to the scheme illustrated in **Figure 2**.

Example 1

8-Methoxy-1-(methylsulfonyl)oxy-3,6-dioxaoctane (9)

A solution of non-polydispersed triethylene glycol monomethyl ether molecules (4.00 mL, 4.19 g, 25.5 mmol) and triethylamine (4.26 mL, 3.09 g, 30.6 mmol) in dry dichloromethane (50 mL) was chilled in an ice bath and place under a nitrogen atmosphere. A solution of methanesulfonyl chloride (2.37 mL, 3.51 g, 30.6 mmol) in dry dichloromethane (20 mL) was added dropwise from an addition funnel. Ten minutes after the completion of the chloride addition, the reaction mixture was removed from the ice bath and allowed to come to room temperature. The mixture was stirred for an additional hour, at which time TLC ($CHCl_3$ with 15% MeOH as the elutant) showed no remaining triethylene glycol monomethyl ether.

The reaction mixture was diluted with another 75 mL of dichloromethane and washed successively with saturated $NaHCO_3$, water and brine. The organics were dried over Na_2SO_4, filtered and concentrated in vacuo to give a non-polydispersed mixture of compounds **9** as a clear oil (5.31g, 86%).

Example 2

Ethylene glycol mono methyl ether (10) (m=4,5,6)

To a stirred solution of non-polydispersed compound **11** (35.7 mmol) in dry DMF (25.7 mL), under N_2 was added in portion a 60% dispersion of NaH in mineral oil, and the mixture was stirred at room temperature for 1 hour. To this salt **12** was added a solution of non-polydispersed mesylate **9** (23.36) in dry DMF (4 ml) in a single portion, and the mixture was stirred at room temperature for 3.5 hours. Progress of the reaction was monitored by TLC (12% CH_3OH-$CHCl_3$). The reaction mixture was diluted with an equal amount of 1N HCl, and extracted with ethyl acetate (2 x 20 ml) and discarded. Extraction of aqueous solution and work-up gave non-polydispersed polymer **10** (82 -84% yield).

Example 3

3,6,9,12,15,18,21-Heptaoxadocosanol (10) (m=4)

Oil; Rf 0.46 (methanol : chloroform = 3:22); MS m/z calc'd for $C_{15}H_{32}O_8$ 340.21 (M^++1), found 341.2.

Example 4

3,6,9,12,15,18,21,24-Octaoxapentacosanol (10) (m=5)

Oil; Rf 0.43 (methanol : chloroform = 6:10); MS m/z calc'd for $C_{17}H_{36}O_9$ 384.24 (M^++1), found 385.3.

Example 5

3,6,9,12,15,18,21,24,27-Nonaoxaoctacosanol (10) (m=6)

Oil; Rf 0.42 (methanol : chloroform = 6:10); MS m/z calc'd for $C_{19}H_{40}O_{10}$ 428.26 (M^++1), found 429.3.

Example 6

20-methoxy-1-(methylsulfonyl)oxy-3,6,9,12,15,18-hexaoxaeicosane (14)

Non-polydispersed compound **14** was obtained in quantitative yield from the alcohol **13** (m=4) and methanesulfonyl chloride as described for **9**, as an oil; Rf 0.4 (ethyl acetate : acetonitrile = 1:5); MS m/z calc'd for $C_{17}H_{37}O_{10}$ 433.21 (M^++1), found 433.469.

Example 7

Ethylene glycol mono methyl ether (15) (m=3,4,5)

The non-polydispersed compounds **15** were prepared from a diol by using the procedure described above for compound **10**.

Example 8

3,6,9,12,15,18,21,24,27,30-Decaoxaheneicosanol (15) (m=3)

Oil; Rf 0.41 (methanol : chloroform = 6:10); MS m/z calc'd for $C_{21}H_{44}O_{11}$ 472.29 (M^{+}+1), found 472.29.

Example 9

3,6,9,12,15,18,21,24,27,30,33-Unecaoxatetratricosanol (15) (m=4)

Oil; Rf 0.41 (methanol : chloroform = 6:10); MS m/z calc'd for $C_{23}H_{48}O_{12}$ 516.31 (M^{+}+1), found 516.31.

Example 10

3,6,9,12,15,18,21,24,27,30,33,36-Dodecaoxaheptatricosanol (15) (m=5)

Oil; Rf 0.41 (methanol : chloroform = 6:10); MS m/z calc'd for $C_{25}H_{52}O_{13}$ 560.67 (M^{+}+1), found 560.67.

Examples 11 through 18 refer to the scheme illustrated in **Figure 3**.

Example 11

Hexaethylene glycol monobenzyl ether (16)

An aqueous sodium hydroxide solution prepared by dissolving 3.99 g (100 mmol) NaOH in 4 ml water was added slowly to non-polydispersed hexaethylene glycol (28.175 g, 25 ml, 100 mmol). Benzyl chloride (3.9 g, 30.8 mmol, 3.54 ml) was added and the reaction mixture was heated with stirring to 100°C for 18 hours. The reaction mixture was then cooled, diluted with brine (250 ml) and extracted with methylene chloride (200 ml x 2). The combined organic layers were washed with brine once, dried over Na_2SO_4, filtered and concentrated in vacuo to a dark brown oil. The crude product mixture was purified *via* flash

chromatography (silica gel, gradient elution: ethyl acetate to 9/1 ethyl acetate/methanol) to yield 8.099 g (70 %) of non-polydispersed **16** as a yellow oil.

Example 12

Ethyl 6-methylsulfonyloxyhexanoate (17)

A solution of non-polydispersed ethyl 6-hydroxyhexanoate (50.76 ml, 50.41 g, 227 mmol) in dry dichloromethane (75 ml) was chilled in a ice bath and placed under a nitrogen atmosphere. Triethylamine (34.43 ml, 24.99 g, 247 mmol) was added. A solution of methanesulfonyl chloride (19.15 ml, 28.3 g, 247 mmol) in dry dichloromethane (75 ml) was added dropwise from an addition funnel. The mixture was stirred for three and one half hours, slowly being allowed to come to room temperature as the ice bath melted. The mixture was filtered through silica gel, and the filtrate was washed successively with water, saturated $NaHCO_3$, water and brine. The organics were dried over Na_2SO_4, filtered and concentrated in vacuo to a pale yellow oil. Final purification of the crude product was achieved by flash chromatography (silica gel, 1/1 hexanes/ethyl acetate) to give the non-polydispersed product (46.13 g, 85 %) as a clear, colorless oil. FAB MS: *m/e* 239 (M+H), 193 ($M-C_2H_5O$).

Example 13

6-{2-[2-(2-{2-[2-(2-Benzyloxyethoxy)ethoxy]ethoxy}-ethoxy)-ethoxy]-ethoxy}-hexanoic acid ethyl ester (18)

Sodium hydride (3.225 g or a 60 % oil dispersion, 80.6 mmol) was suspended in 80 ml of anhydrous toluene, placed under a nitrogen atmosphere and cooled in an ice bath. A solution of the non-polydispersed alcohol **16** (27.3 g, 73.3 mmol) in 80 ml dry toluene was added to the NaH suspension. The mixture was stirred at 0°C for thirty minutes, allowed to come to room temperature and stirred for another five hours, during which time the mixture became a clear brown solution. The non-polydispersed mesylate **17** (19.21 g, 80.6 mmol) in 80 ml dry toluene was added to the NaH/alcohol mixture, and the combined solutions were stirred at room temperature for three days. The reaction mixture was quenched with 50 ml methanol and filtered through basic alumina. The filtrate was concentrated in vacuo and purified by flash chromatography (silica gel, gradient elution: 3/1 ethyl acetate/hexanes to

ethyl acetate) to yield the non-polydispersed product as a pale yellow oil (16.52 g, 44 %). FAB MS: *m/e* 515 (M+H).

Example 14

6-{2-[2-(2-{2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}-ethoxy)-ethoxy]-ethoxy}-hexanoic acid ethyl ester (19)

Non-polydispersed benzyl ether **18** (1.03 g, 2.0 mmol) was dissolved in 25 ml ethanol. To this solution was added 270 mg 10 % Pd/C, and the mixture was placed under a hydrogen atmosphere and stirred for four hours, at which time TLC showed the complete disappearance of the starting material. The reaction mixture was filtered through Celite 545 to remove the catalyst, and the filtrate was concentrated in vacuo to yield the non-polydispersed title compound as a clear oil (0.67 g, 79 %). FAB MS: *m/e* 425 (M+H), 447 (M+Na).

Example 15

6-{2-[2-(2-{2-[2-(2-methylsulfonylethoxy)ethoxy]ethoxy}-ethoxy)-ethoxy]-ethoxy}-hexanoic acid ethyl ester (20)

The non-polydispersed alcohol **19** (0.835 g, 1.97 mmol) was dissolved in 3.5 ml dry dichloromethane and placed under a nitrogen atmosphere. Triethylamine (0.301 ml, 0.219 g, 2.16 mmol) was added and the mixture was chilled in an ice bath. After two minutes, the methanesulfonyl chloride (0.16 ml, 0.248 g, 2.16 mmol) was added. The mixture was stirred for 15 minutes at $0^{o}C$, then at room temperature for two hours. The reaction mixture was filtered through silica gel to remove the triethylammonium chloride, and the filtrate was washed successively with water, saturated $NaHCO_3$, water and brine. The organics were dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by column chromatography (silica gel, 9/1 ethyl acetate/methanol) to give non-polydispersed compound **20** as a clear oil (0.819 g, 83 %). FAB MS: *m/e* 503 (M+H).

Example 16

6-(2-{2-[2-(2-{2-[2-(2-methoxyethoxy)ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-ethoxy)-hexanoic acid ethyl ester (21)

NaH (88 mg of a 60 % dispersion in oil, 2.2 mmol) was suspended in anhydrous

toluene (3 ml) under N_2 and chilled to 0 ^{o}C. Non-polydispersed diethylene glycol monomethyl ether (0.26 ml, 0.26 g, 2.2 mmol) that had been dried *via* azeotropic distillation with toluene was added. The reaction mixture was allowed to warm to room temperature and stirred for four hours, during which time the cloudy grey suspension became clear and yellow and then turned brown. Mesylate **20** (0.50 g, 1.0 mmol) in 2.5 ml dry toluene was added. After stirring at room temperature over night, the reaction was quenched by the addition of 2 ml of methanol and the resultant solution was filtered through silica gel. The filtrate was concentrated in vacuo and the FAB MS: *m/e* 499 (M+H), 521 (M+Na). Additional purification by preparatory chromatography (silica gel, 19/3 chloroform/methanol) provided the non-polydispersed product as a clear yellow oil (0.302 g 57 %). FAB MS: *m/e* 527 (M+H), 549 (M+Na).

Example 17

6-(2-{2-[2-(2-{2-[2-(2-methoxyethoxy)ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-ethoxy)-hexanoic acid (22)

Non-polydispersed ester **21** (0.25 g, 0.46 mmol) was stirred for 18 hours in 0.71 ml of 1 N NaOH. After 18 hours, the mixture was concentrated in vacuo to remove the alcohol and the residue dissolved in a further 10 ml of water. The aqueous solution was acidified to pH 2 with 2 N HCl and the product was extracted into dichloromethane (30 ml x 2). The combined organics were then washed with brine (25 ml x 2), dried over Na_2SO_4, filtered and concentrated in vacuo to yield the non-polydispersed title compound as a yellow oil (0.147 g, 62 %). FAB MS: *m/e* 499 (M+H), 521 (M+Na).

Example 18

6-(2-{2-[2-(2-{2-[2-(2-methoxyethoxy)ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-ethoxy)-hexanoic acid 2,5-dioxo-pyrrolidin-1-yl ester (23)

Non-polydispersed acid **22** (0.209 g, 0.42 mmol) were dissolved in 4 ml of dry dichloromethane and added to a dry flask already containing NHS (N-hydroxysuccinimide) (57.8 mg, 0.502 mmol) and EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) (98.0 mg, 0.502 mmol) under a N_2 atmosphere. The solution was stirred at room temperature overnight and filtered through silica gel to remove excess reagents and the urea formed from the EDC. The filtrate was concentrated in vacuo to provide the non-

polydispersed product as a dark yellow oil (0.235 g, 94 %). FAB MS: *m/e* 596 (M+H), 618 (M+Na).

Examples 19 through 24 refer to the scheme illustrated in **Figure 4**.

Example 19

Mesylate of triethylene glycol monomethyl ether (24)

To a solution of CH_2Cl_2 (100 mL) cooled to 0°C in an ice bath was added non-polydispersed triethylene glycol monomethyl ether (25 g, 0.15 mol). Then triethylamine (29.5 mL, 0.22 mol) was added and the solution was stirred for 15 min at 0°C, which was followed by dropwise addition of methanesulfonyl chloride (13.8 mL, 0.18 mol, dissolved in 20 mL CH_2Cl_2). The reaction mixture was stirred for 30 min at 0°C, allowed to warm to room temperature, and then stirred for 2 h. The crude reaction mixture was filtered through Celite (washed CH_2Cl_2 ~200 mL), then washed with H_2O (300 mL), 5% $NaHCO_3$ (300 mL), H_2O (300 mL), sat. NaCl (300 mL), dried $MgSO_4$, and evaporated to dryness. The oil was then placed on a vacuum line for ~2h to ensure dryness and afforded the non-polydispersed title compound as a yellow oil (29.15 g, 80% yield).

Example 20

Heptaethylene glycol monomethyl ether (25)

To a solution of non-polydispersed tetraethylene glycol (51.5 g, 0.27 mol) in THF (1L) was added potassium t-butoxide (14.8 g, 0.13 mol, small portions over ~30 min). The reaction mixture was then stirred for 1h and then **24** (29.15 g, 0.12 mol) dissolved in THF (90 mL) was added dropwise and the reaction mixture was stirred overnight. The crude reaction mixture was filtered through Celite (washed CH_2Cl_2, ~200 mL) and evaporated to dryness. The oil was then dissolved in HCl (250 mL, 1 N) and washed with ethyl acetate (250 mL) to remove excess **24**. Additional washings of ethyl acetate (125 mL) may be required to remove remaining **24**. The aqueous phase was washed repetitively with CH_2Cl_2 (125 mL volumes) until most of the **25** has been removed from the aqueous phase. The first extraction will contain **24**, **25**, and dicoupled side product and should be back extracted with HCl (125 mL, 1N). The organic layers were combined and evaporated to dryness. The resultant oil was then dissolved in CH_2Cl_2 (100 mL) and washed repetitively with H_2O (50 mL volumes) until

25 was removed. The aqueous fractions were combined, total volume 500 mL, and NaCl was added until the solution became cloudy and then was washed with CH_2Cl_2 (2 x 500 mL). The organic layers were combined, dried $MgSO_4$, and evaporated to dryness to afford a the non-polydispersed title compound as an oil (16.9 g, 41% yield). It may be desirable to repeat one or more steps of the purification procedure to ensure high purity.

Example 21

8-Bromooctoanate (26)

To a solution of 8-bromooctanoic acid (5.0 g, 22 mmol) in ethanol (100 mL) was added H_2SO_4 (0.36 mL, 7.5 mmol) and the reaction was heated to reflux with stirring for 3 h. The crude reaction mixture was cooled to room temperature and washed H_2O (100 mL), sat. $NaHCO_3$ (2 x 100 mL), H_2O (100 mL), dried $MgSO_4$, and evaporated to dryness to afford a clear oil (5.5 g, 98% yield).

Example 22

Synthesis of MPEG7-C8 ester (27)

To a solution of the non-polydispersed compound **25** (3.0 g, 8.8 mmol) in ether (90 mL) was added potassium t-butoxide (1.2 g, 9.6 mmol) and the reaction mixture was stirred for 1 h. Then dropwise addition of the non-polydispersed compound **26** (2.4 g, 9.6 mmol), dissolved in ether (10 mL), was added and the reaction mixture was stirred overnight. The crude reaction mixture was filtered through Celite (washed CH_2Cl_2, ~200 mL) and evaporated to dryness. The resultant oil was dissolved in ethyl acetate and washed H_2O (2 x 200 mL), dried $MgSO_4$, and evaporated to dryness. Column chromatography (Silica, ethyl acetate to ethyl acetate/methanol, 10:1) was performed and afforded the non-polydispersed title compound as a clear oil (0.843 g, 19% yield).

Example 23

MPEG7-C8 acid (28)

To the oil of the non-polydispersed compound **27** (0.70 g, 1.4 mmol) was added 1N NaOH (2.0 mL) and the reaction mixture was stirred for 4h. The crude reaction mixture was concentrated, acidified (pH~2), saturated with NaCl, and washed CH_2Cl_2 (2 x 50 mL). The

organic layers were combined, washed sat. NaCl, dried $MgSO_4$, and evaporated to dryness to afford the non-polydispersed title compound as a clear oil (0.35 g, 53% yield).

Example 24

Activation of MPEG7-C8 acid (29)

Non-polydispersed mPEG7-C8-acid **28** (0.31g, 0.64 mmol) was dissolved in 3 ml of anhydrous methylene chloride and then solution of N-hydroxysuccinimide (0.079g, 0.69 mmol) and EDCI·HCl (135.6 mg, 0.71 mmol) in anhydrous methylene chloride added. Reaction was stirred for several hours, then washed with 1N HCl, water, dried over $MgSO_4$, filtered and concentrated. Crude material was purified by column chromatography, concentrated to afford the non-polydispersed title compound as a clear oil and dried *via* vacuum.

Examples 25 through 29 refer to the scheme illustrated in **Figure 5**.

Example 25

10-hydroxydecanoate (30)

To a solution of non-polydispersed 10-hydroxydecanoic acid (5.0 g, 26.5 mmol) in ethanol (100 mL) was added H_2S0_4 (0.43 mL, 8.8 mmol) and the reaction was heated to reflux with stirring for 3 h. The crude reaction mixture was cooled to room temperature and washed H_2O (100 mL), sat. $NaHCO_3$ (2 x 100 mL), H_20 (100 mL), dried $MgSO_4$, and evaporated to dryness to afford the non-polydispersed title compound as a clear oil (6.9 g, 98% yield).

Example 26

Mesylate of 10-hydroxydecanoate (31)

To a solution of CH_2Cl_2 (27 mL) was added non-polydispersed 10-hydroxydecanoate **30** (5.6 g, 26 mmol) and cooled to 0°C in an ice bath. Then triethylamine (5 mL, 37 mmol) was added and the reaction mixture was stirred for 15 min at 0°C. Then methanesulfonyl chloride (2.7 mL, 24 mmol) dissolved in CH_2Cl_2 (3 mL) was added and the reaction mixture was stirred at 0°C for 30 min, the ice bath was removed and the reaction was stirred for an additional 2 h at room temperature. The crude reaction mixture was filtered through Celite

(washed CH_2Cl_2, 80 mL) and the filtrate was washed H_2O (100 mL), 5% $NaHCO_3$ (2 x 100 mL), H_2O (100 mL), sat. NaCl (100 mL), dried $MgSO_4$, and evaporated to dryness to afford the non-polydispersed title compound as a yellowish oil (7.42 g, 97% yield).

Example 27

MPEG$_7$-C$_{10}$ Ester (32)

To a solution of non-polydispersed heptaethylene glycol monomethyl ether **25** (2.5 g, 7.3 mmol) in tetrahydrofuran (100 mL) was added sodium hydride (0.194 g, 8.1 mmol) and the reaction mixture was stirred for 1 h. Then dropwise addition of mesylate of non-polydispersed 10-hydroxydecanoate **31** (2.4 g, 8.1 mmol), dissolved in tetrahydrofuran (10 mL), was added and the reaction mixture was stirred overnight. The crude reaction mixture was filtered through Celite (washed CH_2Cl_2, ~200 mL) and evaporated to dryness. The resultant oil was dissolved in ethyl acetate and washed H_2O (2 x 200 mL), dried $MgSO_4$, evaporated to dryness, chromatographed (silica, ethyl acetate/methanol, 10:1), and chromatographed (silica, ethyl acetate) to afford the non-polydispersed title compound as a clear oil (0.570 g, 15% yield).

Example 28

MPEG$_7$-C$_{10}$ Acid (33)

To the oil of non-polydispersed mPEG$_7$-C$_{10}$ ester **32** (0.570 g, 1.1 mmol) was added 1N NaOH (1.6 mL) and the reaction mixture was stirred overnight. The crude reaction mixture was concentrated, acidified (pH~2), saturated with NaCl, and washed CH_2Cl_2 (2 x 50 mL). The organic layers were combined, washed sat. NaCl (2 x 50 mL), dried $MgSO_4$, and evaporated to dryness to afford the non-polydispersed title compound as a clear oil (0.340 g, 62% yield).

Example 29

Activation of MPEG$_7$-C$_{10}$ Acid (34)

The non-polydispersed acid **33** was activated using procedures similar to those described above in Example 24.

Examples 30 and 31 refer to the scheme illustrated in **Figure 6**.

Example 30

Synthesis of C18(PEG6) Oligomer (36)

Non-polydispersed stearoyl chloride **35** (0.7g, 2.31 mmol) was added slowly to a mixture of PEG6 (5 g, 17.7 mmol) and pyridine (0.97g, 12.4 mmol) in benzene. The reaction mixture was stirred for several hours (~5). The reaction was followed by TLC using ethylacetate/methanol as a developing solvent. Then the reaction mixture was washed with water, dried over $MgSO_4$, concentrated and dried *via* vacuum. Purified non-polydispersed compound **36** was analyzed by FABMS: m/e 549/ M^+H.

Example 31

Activation of C18(PEG6) Oligomer

Activation of non-polydispersed C18(PEG6) oligomer was accomplished in two steps:

1) Non-polydispersed stearoyl-PEG6 **36** (0.8 g, 1.46 mmol) was dissolved in toluene and added to a phosgene solution (10 ml, 20 % in toluene) which was cooled with an ice bath. The reaction mixture was stirred for 1 h at $0^{\circ}C$ and then for 3 h at room temperature. Then phosgene and toluene were distilled off and the remaining non-polydispersed stearoyl PEG6 chloroformate **37** was dried over P_2O_5 overnight.

2) To a solution of non-polydispersed stearoyl PEG6 chloroformate **36** (0.78g, 1.27 mmol) and TEA (128 mg, 1.27 mmol) in anhydrous methylene chloride, N-hydroxy succinimide (NHS) solution in methylene chloride was added. The reaction mixture was stirred for 16 hours, then washed with water, dried over $MgSO_4$, filtered, concentrated and dried *via* vacuum to provide the non-polydispersed activated C18(PEG6) oligomer **38**.

Examples 32 through 37 refer to the scheme illustrated in **Figure 7**.

Example 32

Tetraethylene glycol monobenzylether (39)

To the oil of non-polydispersed tetraethylene glycol (19.4 g, 0.10 mol) was added a solution of NaOH (4.0 g in 4.0 mL) and the reaction was stirred for 15 mm. Then benzyl chloride (3.54 mL, 30.8 mmol) was added and the reaction mixture was heated to 100°C and

stirred overnight. The reaction mixture was cooled to room temperature, diluted with sat. NaCl (250 mL), and washed CH_2Cl_2 (2 x 200 mL). The organic layers were combined, washed sat. NaCl, dried $MgSO_4$, and chromatographed (silica, ethyl acetate) to afford the non-polydispersed title compound as a yellow oil (6.21 g, 71% yield).

Example 33

Mesylate of tetraethylene glycol monobenzylether (40)

To a solution of CH_2Cl_2 (20 mL) was added non-polydispersed tetraethylene glycol monobenzylether **39** (6.21 g, 22 mmol) and cooled to 0°C in an ice bath. Then triethylamine (3.2 mL, 24 mmol) was added and the reaction mixture was stirred for 15 min at 0°C. Then methanesulfonyl chloride (1.7 mL, 24 mmol) dissolved in CH_2Cl_2 (2 mL) was added and the reaction mixture was stirred at 0°C for 30 min, the ice bath was removed and the reaction was stirred for an additional 2 h at room temperature. The crude reaction mixture was filtered through Celite (washed CH_2Cl_2, 80 mL) and the filtrate was washed H_2O (100 mL), 5% $NaHCO_3$ (2 x 100 mL), H_20 (100 mL), sat. NaCl (100 mL), and dried $MgSO_4$. The resulting yellow oil was chromatographed on a pad of silica containing activated carbon (10 g) to afford the non-polydispersed title compound as a clear oil (7.10 g, 89% yield).

Example 34

Octaethylene glycol monobenzylether (41)

To a solution of tetrahydrofuran (140 mL) containing sodium hydride (0.43 g, 18 mmol) was added dropwise a solution of non-polydispersed tetraethylene glycol (3.5 g, 18 mmol) in tetrahydrofuran (10 mL) and the reaction mixture was stirred for 1 h. Then mesylate of non-polydispersed tetraethylene glycol monobenzylether **40** (6.0 g, 16.5 mmol) dissolved in tetrahydrofuran (10 mL) was added dropwise and the reaction mixture was stirred overnight. The crude reaction mixture was filtered through Celite (washed, CH_2Cl_2, 250 mL) and the filtrate was washed H_2O, dried $MgSO_4$, and evaporated to dryness. The resultant oil was chromatographed (silica, ethyl acetate/methanol, 10:1) and chromatographed (silica, chloroform/methanol, 25:1) to afford the non-polydispersed title compound as a clear oil (2.62 g, 34% yield).

Example 35

Synthesis of Stearate PEG8-Benzyl (43)

To a stirred cooled solution of non-polydispersed octaethylene glycol monobenzylether **41** (0.998 g, 2.07 mmol) and pyridine (163.9 mg, 2.07 mmol) was added non-polydispersed stearoyl chloride **42** (627.7 mg, 2.07 mmol) in benzene. The reaction mixture was stirred overnight (18 hours). The next day the reaction mixture was washed with water, dried over $MgSO_4$, concentrated and dried *via* vacuum. Then the crude product was chromatographed on flash silica gel column, using 10% methanol/90% chloroform. The fractions containing the product were combined, concentrated and dried *via* vacuum to afford the non-polydispersed title compound.

Example 36

Hydrogenolysis of Stearate-PEG8-Benzyl

To a methanol solution of non-polydispersed stearate-PEG8-Bzl **43** (0.854g 1.138 mmol) Pd/C(10%) (palladium, 10% wt. on activated carbon) was added. The reaction mixture was stirred overnight (18 hours) under hydrogen. Then the solution was filtered, concentrated and purified by flash column chromatography using 10% methanol/90% chloroform, fractions with R_f=0.6 collected, concentrated and dried to provide the non-polydispersed acid **44**.

Example 37

Activation of C18(PEG8) Oligomer

Two step activation of non-polydispersed stearate-PEG8 oligomer was performed as described for stearate-PEG6 in Example 31 above to provide the non-polydispersed activated C18(PEG8) oligomer **45**.

Example 38

Synthesis of Activated Triethylene Glycol Monomethyl Oligomers

The following description refers to the scheme illustrated in **Figure 8**. A solution of toluene containing 20% phosgene (100 ml, approximately 18.7 g, 189 mmol phosgene) was chilled to 0°C under a N_2 atmosphere. Non-polydispersed mTEG (triethylene glycol, monomethyl ether, 7.8 g, 47.5 mmol) was dissolved in 25 mL anhydrous ethyl acetate and

added to the chilled phosgene solution. The mixture was stirred for one hour at 0°C, then allowed to warm to room temperature and stirred for another two and one half hours. The remaining phosgene, ethyl acetate and toluene were removed *via* vacuum distillation to leave the non-polydispersed mTEG chloroformate **46** as a clear oily residue.

The non-polydispersed residue **46** was dissolved in 50 mL of dry dichloromethane to which was added TEA (triethyleamine, 6.62 mL, 47.5 mmol) and NHS (N-hydroxysuccinimide, 5.8 g, 50.4 mmol). The mixture was stirred at room temperature under a dry atmosphere for twenty hours during which time a large amount of white precipitate appeared. The mixture was filtered to remove this precipitate and concentrated *in vacuo*. The resultant oil **47** was taken up in dichloromethane and washed twice with cold deionized water, twice with 1N HCl and once with brine. The organics were dried over $MgSO_4$, filtered and concentrated to provide the non-polydispersed title compound as a clear, light yellow oil. If necessary, the NHS ester could be further purified by flash chromatography on silica gel using EtOAc as the elutant.

Example 39

Synthesis of Activated Palmitate-TEG Oligomers

The following description refers to the scheme illustrated in **Figure 9**. Non-polydispersed palmitic anhydride (5 g; 10 mmol) was dissolved in dry THF (20 mL) and stirred at room temperature. To the stirring solution, 3 mol excess of pyridine was added followed by non-polydispersed triethylene glycol (1.4 mL). The reaction mixture was stirred for 1 hour (progress of the reaction was monitored by TLC; ethyl acetate-chloroform; 3:7). At the end of the reaction, THF was removed and the product was mixed with 10% H_2SO_4 acid and extracted ethyl acetate (3 x 30 mL). The combined extract was washed sequentially with water, brine, dried over $MgSO_4$, and evaporated to give non-polydispersed product **48**. A solution of N,N'-disuccinimidyl carbonate (3 mmol) in DMF (~10 mL) is added to a solution of the non-polydispersed product **48** (1 mmol) in 10 mL of anydrous DMF while stirring. Sodium hydride (3 mmol) is added slowly to the reaction mixture. The reaction mixture is stirred for several hours (e.g., 5 hours). Diethyl ether is added to precipitate the activated oligomer. This process is repeated 3 times and the product is finally dried.

Example 40

Synthesis of Human Growth Hormone-Oligomer Conjugates with Activated Hexaethylene Glycol Monomethyl Oligomers

The following description refers to the scheme illustrated in **Figure 10**. Non-polydispersed activated hexaethylene glycol monomethyl ether was prepared analogously to that of non-polydispersed triethylene glycol in Example 38 above. A 20% phosgene in toluene solution (35 mL, 6.66 g, 67.4 mmol phosgene) was chilled under a N_2 atmosphere in an ice/salt water bath. Non-polydispersed hexaethylene glycol **50** (1.85 mL, 2.0 g, 6.74 mmol) was dissolved in 5 mL anhydrous EtOAc and added to the phosgene solution *via* syringe. The reaction mixture was kept stirring in the ice bath for one hour, removed and stirred a further 2.5 hours at room temperature. The phosgene, EtOAc, and toluene were removed by vacuum distillation, leaving non-polydispersed compound **51** as a clear, oily residue.

The non-polydispersed residue **51** was dissolved in 20 mL dry dichloromethane and placed under a dry, inert atmosphere. Triethylamine (0.94 mL, 0.68 g, 6.7 mmol) and then NHS (N-hydroxy succinimide, 0.82 g, 7.1 mmol) were added, and the reaction mixture was stirred at room temperature for 18 hours. The mixture was filtered through silica gel to remove the white precipitate and concentrated *in vacuo*. The residue was taken up in dichloromethane and washed twice with cold water, twice with 1 N HCl and once with brine. The organics were dried over Na_2SO_4, filtered and concentrated. Final purification was done via flash chromatography (silica gel, EtOAc) to obtain the UV active non-polydispersed NHS ester **52**.

Human growth hormone (somatropin (rDNA origin) for injection), available under the trade name Saizen™ from Serono of Randolph, Massachusetts, was dissolved in DMSO such that the hGH was at a 0.58 mmol concentration. TEA (278 equivalents) was added and the solution was stirred for approximately ten minutes. Two equivalents, five equivalents or thirty equivalents of non-polydispersed activated hexaethylene glycol **52** was added from a 0.2 M solution of the activated oligomer in dry THF. Reactions were stirred at room temperature for 45 minutes to one hour. Aliquots of each reaction mixture were quenched in 600 μL of 0.1% TFA in water. HPLC comparison of the 2 polymer equivalent and the 5 polymer equivalent reaction mixtures vs. unconjugated hGH is shown in **Figure 14**. HPLC analysis of the thirty polymer equivalent reaction is shown in **Figure 15**.

Samples of the conjugates for mass spectroscopy were purified *via* analytical HPLC using a reversed-phase C_{18} column and a water/acetonitrile gradient. The entire peak from the 2 equivalent reaction mixture was collected, concentrated and analyzed using MALDI mass spectroscopy. The mass spectra of this material showed evidence of the presence of mono-conjugated, di-conjugated, tri-conjugated and tetra-conjugated hGH as well as some remaining unreacted hGH (**Figure 16**). The five equivalent reaction mixture was purified crudely according to polarity as indicated in **Figure 17**. MALDI mass spectra of the concentrated fractions (**Figure 18**, **Figure 19** and **Figure 20**) indicated that the level of conjugation of the protein increased with retention time. Electrospray mass spectra of fraction E, **Figure 21**, gave results consistent with the presence of hexa-conjugated hGH. The entire peak from the thirty polymer equivalent reaction mixture was collected and concentrated. Electrospray mass spectral analysis, **Figure 22**, showed deca- and higher conjugated material.

A similar procedure is used to conjugate hGH with the activated oligomer of Example 18, 24, 29, 31 or 37.

Example 41

Synthesis of Human Growth Hormone-Oligomer Conjugates with Activated Palmitate-TEG Oligomers

Procedures similar to those described above in Example 41 were performed using the activated polymer from Example 39. Progress of conjugation was checked by HPLC by taking 20 μL of the conjugated reaction mixture in a vial and diluting with 100 μL of 0.1% TFA-water-IPA (1:1), the results of which are illustrated in **Figure 23**. After 2 hours, the reaction was quenched by adding 0.1% TFA-water. The conjugated product was purified by prep. HPLC.

Example 42

Synthesis of Human Growth Hormone-Oligomer Conjugates with Activated TEG Oligomers

One equivalent of human growth hormone (hGH) (somatropin (rDNA origin) for injection), available under the trade name Saizen™ from Serono of Randolph, Massachusetts was dissolved in DMSO (1 mg/125 μL) and stirred at room temperature for 2-4 minutes. Two equivalents TEA was added followed by two equivalents of the activated oligomer of

Example 39, which was dissolved in THF. After 2 hours, the reaction was quenched by adding 0.1% TFA-water. The conjugated product was purified by prep. HPLC as illustrated in **Figure 24**.

A similar procedure five equivalents TEA and five equivalents of the activated oligomer of Example 39 was performed. The conjugated product was purified by prep. HPLC using C18 column as illustrated in **Figure 25**. The mobile phase and elution time were as follows:

Time	mL/min	Solvent A	Solvent B
0	3.5	80	20
55	3.5	0	100

The pooled fraction was lyophilized into a white powder. The mass spectra for the compound are illustrated in **Figures 26** and **27**.

A similar procedure utilizing nine equivalents TEA and nine equivalents of the activated oligomer of Example 39 was performed. The conjugated product was purified by prep. HPLC using C18 column as illustrated in **Figure 28**.

Example 43

Determination of the Dispersity Coefficient for a Mixture of Human Growth Hormone-Oligomer Conjugates

The dispersity coefficient of a mixture of human growth hormone-oligomer conjugates is determined as follows. A mixture of human growth hormone-oligomer conjugates is provided, for example, as described above in Example 40. A first sample of the mixture is purified via HPLC to separate and isolate the various human growth hormone-oligomer conjugates in the sample. Assuming that each isolated fraction contains a purely monodispersed mixture of conjugates, "n" is equal to the number of fractions collected. The mixture may include one or more conjugates, including mono-, di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, and/or nona-conjugates. Each isolated fraction of the mixture is analyzed via mass spectroscopy to determine the mass of the fraction, which allows each isolated fraction to be categorized by its degree of conjugation and provides a value for the variable "M_i" for each conjugate in the sample.

A second sample of the mixture is analyzed via HPLC to provide an HPLC trace. Assuming that the molar absorptivity does not change as a result of the conjugation, the

weight percent of a particular conjugate in the mixture is provided by the area under the peak of the HPLC trace corresponding to the particular conjugate as a percentage of the total area under all peaks of the HPLC trace. The sample is collected and lyophilized to dryness to determine the anhydrous gram weight of the sample. The gram weight of the sample is multiplied by the weight percent of each component in the sample to determine the gram weight of each conjugate in the sample. The variable "N_i" is determined for a particular conjugate (the i^{th} conjugate) by dividing the gram weight of the particular conjugate in the sample by the mass of the particular conjugate and multiplying the quotient by Avagadro's number (6.02205×10^{23} $mole^{-1}$), M_i, determined above, to give the number of molecules of the particular conjugate, N_i, in the sample. The dispersity coefficient is then calculated using n, M_i as determined for each conjugate, and N_i as determined for each conjugate.

Example 44

An assay was performed as follows:

Cell culture: Stable clones expressing the full length human GHR were generated in 293 cells (human kidney embryonal cell line), designated 293GHR, as previously described.

Transcription assays: These were performed in 293 GHR cells transiently transfected with a reported construct containing a Stat5-binding element (LHRE) fused to a minimal TK promoter and luciferase. A β-galactosidase expression vector was co-transfected as a transfection control and luciferase values corrected for β-galactosidase activity. Sixteen hours after transfection, cells were transferred into serum free medium and treated with GH or agonist for 6 hours. Luciferase activity is reported as percentage of maximal activity stimulated by GH in the specific experiment to allow comparison between repeated experiments. The maximal activity stimulated by GH is the fold induction stimulated by GH, i.e. corrected luciferase value in GH stimulated cells divided by corrected luciferase value in unstimulated cells. Results of the assay are shown in **Figures 29** and **30** where Genotropin is human growth hormone (standard, not part of the present invention), GH-002 is a 2 equivalent mTEG conjugate, GH-003 is a 5 equivalent mTEG conjugate, GH-004 is a 5 equivalent mTEG conjugate , Prot hGH is human growth hormone (standard, not part of the present invention), and hGH-TEG is a 9 equivalent mTEG conjugate.

In the specification, there has been disclosed typical preferred embodiments of the invention and, although specific terms are employed, they are used in a generic and descriptive sense only and not for purposes of limitation, the scope of the invention being set forth in the following claims.